THE EPIDEMIOLOGY OF AGE AT NATURAL MENOPAUSE

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Abstract

Background: The menopausal transition may affect women's quality of life. Hormone therapy (HT) has been shown to be effective in alleviating menopausal symptoms, such as hot flashes. The age at natural menopause (ANM) is of clinical relevance since earlier menopause is associated with increased morbidity and mortality. It is therefore important to identify means to ensure a healthy menopause. More than a decade ago HT use rates plummeted, however the current situation on its use is unclear. Factors associated with ANM and HT use vary across populations; little is known on such factors in Canada. Obesity after middle age has deleterious health consequences; yet the relationship between absolute and relative weight, BMI and waist circumference (WC), and ANM is inconclusive.

Objectives: 1) To investigate the role of changes in anthropometric measures on ANM. 2) To elucidate factors associated with ANM in Canada, and 3) to examine the prevalence and characteristics of HT use.

Methods: To answer objective 1, data from the Coronary Artery Risk Development in Young Adults (CARDIA) study were used. To answer objectives 2 and 3, secondary data analysis was conducted on baseline data from the Canadian Longitudinal Study on Aging (CLSA). Multinomial logistic, and Cox Proportional hazard regressions were performed.

Results: Baseline WC and > 5 % loss of baseline weight were modest predictors of ANM in middle-aged women. Premenopausal hypertension and blood pressure indicators were also significantly related to an earlier ANM. Median ANM was 51 years. Having no partner, low household income and education levels, current and former smoking, and cardiovascular disease (CVD) were associated with an earlier ANM, while current employment, alcohol consumption, and obesity were associated with later ANM. Current HT use was 9.5%. Older age, non-white ethnic background, current employment, regular smoking, obesity, and breast cancer were negatively associated with current HT use. Alcohol consumption and presence of allergies or mood disorders were positively associated with current HT use.

Conclusion: Menopausal age is in part determined by modifiable factors. These findings may be used to inform opportunities regarding menopause management, and help in prevention, assessment, and early management of chronic disease risk during the menopausal transition.

Keywords: Age at Menopause, Hormone Therapy, Canada, Factors, Obesity, Aging

Dedication

To my parents: Hagop and Shafika.

In memory of my grandparents.

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Preface

Chapter 2 will be submitted for publication in *Human Reproduction*.

Chapters 3 and 4 are published in *Menopause*, the Journal of the North American Menopause Society.

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List of Abbreviations

AMH: Anti-Mullerian Hormone

ANM: Age at Natural Menopause

BMI: Body mass index

CARDIA: Coronary Artery Risk Development

CLSA: Canadian Longitudinal Study on Aging

CI: Confidence intervals

CVD: Cardiovascular disease

HDL-C: High-density lipoprotein cholesterol

HR: Hazard ratio

HT: Hormone Therapy

LDL-C: Low-density lipoprotein cholesterol

MetS: Metabolic Syndrome

OC: Oral contraceptive

OR: Odds ratio

TG: Triglycerides

VMS: Vasomotor symptoms

WC: Waist circumference

WHO: World Health Organization

WHR: Waist to hip ratio

Chapter 1

General Introduction

Biology of Menopause

The female adult life cycle can be divided into premenopausal, perimenopausal (menopausal transition), and menopausal (postmenopausal) phases[1]. Follicles are the functional units of the ovary that each contains an oocyte and its surrounding granulosa cells. These follicles are necessary for fertility and constitute a woman's "ovarian reserve" which is formed at birth[1]. Given that a woman is born with an exhaustible and non-replenish-able supply of oocytes, the peak number of follicles attained *in utero* or at birth have a major contribution to the age of menopause[2]. The number of follicles peaks at approximately 5 million before birth but then declines to around 2 million at birth. The high rate of loss continues throughout the pubertal years such that only 300,000-400,000 oocytes remain at puberty at which time the rate of loss slows until hormonal changes along with genetic and environmental exposures interact to transition into menopause[3, 4].

Definition of Age at Natural Menopause (ANM)

According to the World Health Organization (WHO) [5], natural menopause is defined as at least 12 consecutive months of amenorrhea not due to surgery or any obvious cause. Natural menopause results from the loss of ovarian follicular function and atresia[3] and is reached when a woman's follicular reserve is depleted to approximately 1000 follicles. Therefore, reproductive aging in women is characterized by a decline in both the quantity and quality of oocytes. Several studies emphasize the major individual variability on the extent of follicle pool depletion during life[6]. Numerous studies revealed a considerable variation in the age at menopause both within and between populations[7-15], approximately 40% to 60% of which is genetically determined [16-18]. Physiological sources of variation in age at menopause include the original number of oocytes and the rates of atresia[19-20].

Outcomes of ANM

Age at menopause has subsequent health implications for women. Later menopausal age increases the risk of breast, endometrial, and ovarian cancers while earlier age of menopause is a known risk factor for the metabolic syndrome (MetS), hypertension, cardiovascular disease

(CVD), atherosclerosis, stroke, autoimmune disease and osteoporosis, due to an earlier deficiency in estrogen[3, 25, 26]. ANM has also been linked to mortality with all-cause mortality being reduced by 2% with each increasing year of menopause[27].

Factors Associated with ANM

The timing of natural menopause may also serve as a marker for reproductive aging with an earlier menopause indicating premature aging[24]. Genetic factors are key factors that influence menopausal age[18]. However, the specific genetic determinants involved remain unclear. Studies suggest that between 15 and 70% of the variability in age of onset of menopause is of non-genetic origin and therefore potentially modifiable[26]. Environmental influences on age at menopause include parity, life-course characteristics, socioeconomic status and health behaviors[21-23]. However, only smoking status is consistently linked to earlier menopause[3, 25, 26]. Some studies have reported a positive association between BMI and age of onset of menopause, while others have not found an association[26]. Studies on physical activity are also inconclusive[27, 28], and the relationship between diet and onset of menopause is inconsistent. For diet, it has been reported that vegetarians undergo an earlier onset of menopause compared to omnivores[29], yet others have found that high consumption of green/ yellow vegetables[28], and fruit[27] is associated with a later menopause. High fat intake was associated with a later onset of menopause in a German study[31], yet no relationship was noted between fat intake and menopause in other studies[27, 28].

Chronic diseases have also been linked to menopause onset[32-34]. The relationship and direction between CVD, and its risk factors in relation to ANM is not clear, however. CVD could be a risk factor for ANM, due to estrogen depletion increasing cardiovascular risk[35]. CVD risk factors such as cholesterol, low-density lipoprotein cholesterol levels, and systolic and diastolic blood pressure levels may be associated with menopausal status, independent of age[36-37].

Menopause and Hormone Therapy (HT)

Menopause is characterized by a series of clinical symptoms, such as vasomotor symptoms (VMS), mood disorders, and poor quality of sleep, all of which are caused by the decline and ultimate cessation of ovarian function[38]. These symptoms may significantly impair the quality of life of women. Hormone therapy (HT) is an appropriate and effective way to

relieve menopause symptoms and some long-term complications that are caused by the decline in endogenous estrogen, such as dyslipidemia, cardiovascular disease and osteoporosis[39-41]. However, the publication of findings from prospective studies, including the Heart and Estrogen/progestin Replacement Study (HERS)[42] and the Women's Health Initiative (WHI) Study[43] raised questions about the safety of HT, particularly with regard to cardiovascular effects and breast cancer. The results of these studies dramatically decreased the world-wide use of HT. Since then, after revision of the initial findings, HT has been shown to have a complex balance of benefits and risks with important effects on various health outcomes, some of which differ according to the various hormone therapy formulations[44]. Few studies have investigated the characteristics of women who use HT. Younger age, higher educational level, postmenopausal status, African American and Hispanic ethnicity, and being married have been associated with HT use, however not all of these associations are consistent[45-50]. Little research has been done on HT use in Canada[51, 52], an ethnically diverse setting where rates of use might differ between subpopulations.

Conceptual Framework

On average, women spend more than a third of their lifetime after the onset of menopause[53]. With the surge in aging populations worldwide, it is estimated that in the year 2030, 1.2 billion women will be peri or postmenopausal and that this total will increase by 4.7 million per year[54]. Life events and experiences are hypothesized to have long lasting influence on health[55]. A life course perspective focuses on how the life history from birth to death of individuals may explain differences in health[55]. Moreover, the life course perspective highlights continuity and changes of exposures across the life span, through trajectories or patterns of behavior over time[56-58]. It is important to note that the term *life span* is embedded in the life-course perspective however; it should be distinguished from the term *life course* since it is seen as a dynamic process in which human development interacts with the social environment in a multilayered social and cultural context[55-58]. Life course epidemiology in particular, attempts to integrate biological risk processes occurring throughout life with disease onset risk and various health and developmental outcomes[59], and therefore, follows the life course perspective. The purpose of life course epidemiology is to build and test models that modulate pathways linking exposures across the life course to health outcomes. According to this perspective, the event of

menopause cannot be separated from ongoing life prior to its occurrence[60]. The life course perspective is embedded in this project, since this framework encourages testing of the temporal order of exposure variables across life and their inter-relationships with the outcome. The life course perspective views menopause as an outcome of many internal (biological) and external factors that interact to control the rate of lifetime follicle loss[61]. Factors which operate earlier in life, such as early life exposures[62], while others such as type 2 diabetes which operate closer to menopause to influence menopause onset, may be integrated within the same model. Specifically, if the associations of earlier factors are to be taken into account, then it is integral to consider other factors across life which may act as confounders or mediators of associations that affect menopause onset.

Significance of the Dissertation

Menopause signals the end of a female's reproductive lifespan, and the timing of this event provides an indication of a woman's reproductive and later physical health. Although age at menopause is a biological marker for aging in women, it is not a pathological condition. Therefore, ensuring means for a healthy menopause, with the term *healthy* indicating overall health from a holistic perspective[60], is crucial. This dissertation aims to develop a model of the factors associated with HT use around the time of menopause as well as age of onset of menopause. Given that the rate of HT use by menopausal women has undergone significant changes in recent years, this dissertation also aims to obtain the prevalence of HT use in the Canadian context. Identifying the prevalence of HT use is important to properly understand whether women are abiding by the guidelines for its use. This in turn can assist in monitoring trends of HT use as well as in aiding future recommendations on its indications. Timing of menopause on the other hand, is a factor in determining total duration of internal estrogen exposure, which portends postmenopausal health risks. Investigating the factors associated with timing of natural menopause will contribute to the detection of women at high risk of developing these health risks. Therefore, describing the correlates of ANM may generate new strategies for improving women's health, as its potential risk factors are amenable to prevention. Second, there is considerable variability in ANM worldwide, and by providing an estimate of the median ANM in Canada, this dissertation will enable international comparisons as well as monitor secular trends in ANM. Examination of exposures across life is essential to understanding potential

critical periods of susceptibility to an earlier or later menopause to identify pathways to menopause. Finally, by examining factors that are potential missing links to understanding age at menopause, this study will lend epidemiologic evidence and methodological insight on novel confounders for future work.

1.1 Specific Objectives

Objective (1): To assess the impact of changes in weight, BMI, and waist circumference across time on ANM in a sample of US women.

Objective (2): To elucidate median ANM and investigate associations between various factors and ANM among Canadian women.

Objective (3): To determine the prevalence of HT use and evaluate the associations between various factors among Canadian women and HT use.

Data from the Canadian Longitudinal Study on Aging (CLSA) and from the Coronary Artery Risk Development in Young Adults (CARDIA) study were used to answer these objectives. The subsequent chapters will present the findings of the individual analyses done to answer the objectives above.

Chapter 2

Longitudinal Changes in Weight, Body Mass Index, Waist Circumference, and Age at Natural Menopause in the Coronary Artery Risk Development (CARDIA) Study

Abstract

Objective: The age at natural menopause (ANM) has subsequent health implications. Earlier ANM is a risk factor for cardiovascular disease (CVD), atherosclerosis, and stroke. Despite extensive study, no clear and conclusive association between anthropometric measures and ANM has emerged. This study aims to assess whether baseline and/or longitudinal changes in anthropometric measures are associated with ANM.

Methods: 2192 premenopausal women from the Coronary Artery Risk Development in Young Adults (CARDIA), a prospective study with 25 years follow up, were included for analysis from 1985-1986 until menopause was attained. Anthropometry included weight, body mass index (BMI) and waist circumference (WC). Absolute and relative changes in anthropometric measures since baseline and each exam were computed. Discrete–time survival analysis was then used to determine the association between anthropometric measures at baseline as well as their absolute and relative changes with ANM, while adjusting for various time-varying and invariant covariates in separate models.

Results: Multivariate Cox regression analysis showed that baseline WC (HR=0.98; 95% CI=0.97-0.99) significantly increased the risk of a later ANM. Neither absolute nor relative changes in anthropometric measures were associated with ANM with one exception. If there was more than 5% weight loss since baseline, the hazard rate for an earlier ANM decreased by 45% (HR=0.55, 95% CI=0.31-0.97). Premenopausal hypertension was strongly associated with an earlier ANM in the model for weight (HR=1.32; 95% CI=1.04-1.66), BMI (HR=1.32; 95% CI=1.05-1.68), and WC (HR=1.35; 95% CI=1.05-1.68), respectively.

Conclusion: These findings show that ANM is partly determined by modifiable factors such as premenopausal hypertension and baseline WC. These results highlight the importance of both control and prevention of cardiovascular risk factors such as excess weight in early to mid-adulthood prior to menopause onset, an independent cardiovascular risk factor.

Keywords: Menopause, Age, Risk Factors, Weight, Waist Circumference, BMI

1.2 Introduction

Age at Natural Menopause (ANM) is of clinical and public health importance as it represents a marker of general health and aging, given that early menopause is associated with higher risk of cardiovascular disease (CVD), cognitive decline, osteoporosis and premature mortality [1,3-8, 10-14]. Despite extensive study, no clear and conclusive association between adiposity (weight and/or BMI) and menopausal age has emerged from the literature. Paramsothy et al[1], using data from the Study of Women's Health Across the Nation (SWAN), found that women with obesity had longer menstrual cycle lengths than women with normal weight during the menopausal transition. Women with longer menstrual cycles during their reproductive years have been shown to have a later age at menopause[2]. In fact, research has suggested, but not consistently[3, 4], that a higher BMI might increase the risk of a later menopause[5-7]. A recent meta-analysis found that increased BMI is moderately associated with a later ANM[8]; however, the studies included were heterogeneous in terms of their study designs and measures of weight. Therefore, the pooled result requires further confirmation. It is hypothesized that increased production of estrone (a precursor of estrogen) in the adipose tissue of women with obesity might contribute to a delay in their menopause[9], as estrone may supplement estradiol levels. Estrone is formed by the peripheral aromatization of androgens, namely androstenedione, secreted by the ovaries and/or adrenal glands[10]. Obesity has been linked to more androgens being converted into estrone[11]. Therefore, increased peripheral production of estrone in women with obesity may contribute to the delay in age at menopause.

It is also suggested that hormonal imbalances occurring as a result of weight change across life increase the rate of follicular atresia[12], thereby influencing the timing of menopause. Therefore, it may be weight change rather than absolute weight per se that influences ANM. However, few studies have examined the relationship between weight change across time and ANM. In fact, previous findings on weight gain or loss and menopausal age were also inconsistent, possibly because of differing methodologies, with studies across various settings showing either no association[13,14], a positive association[7,15,16] or an inverse association[3,12]. Aydin[15] found that premenopausal BMI gain rate and premenopausal weight loss of more than 5 kg were both associated with later ANM. Similarly, Dorjgochoo et al[7] found a dose-response

relationship between midlife weight gain and later ANM. This is in contrast to Kok et al[17] who reported both increasing and decreasing premenopausal relative weight as risk factors for earlier ANM. In a further study, Hardy et al[13] did not find an association between both underweight or overweight BMI trajectories and ANM; however, BMI and age at menopause were self-reported, and BMI was measured from age 18 until 43 years. On the other hand, using data from SWAN, Gold and colleagues[18] found that an increased concurrent BMI and waist circumference were significantly associated with incident vasomotor symptoms (VMS), occurring in early menopause, but a lower VMS risk in late menopause. However, no association was observed between changes in BMI or WC and incident VMS. Moreover, recent analysis from the Nurses' Health Study II by Szegda et al[19] showed that substantial weight loss may increase risk for early natural menopause. Women who lost ≥20 pounds from age 18 to 35 years had an increased odds of early menopause compared to women who gained 5 to 15 pounds. In addition, weight cycling between ages 18 to 30 was also associated with higher risk of early age at natural menopause. Ethnicity, in relation to adiposity and age at menopause, was not assessed due to the majority of the study population being White.

To understand the postmenopausal health implications for both underweight and overweight women, further studies are needed to assess whether absolute weight and weight change play a role on menopausal age. Using a life course approach, determining the effect of changes in adiposity on the timing of menopause is important in order to understand the potential mechanisms by which these changes may act to affect age at menopause, which has been associated with increased risk for various health conditions. Most studies conducted on the effect of the change in adiposity on ANM have, on the whole, relied on recall of early adult weight, and not on objectively assessed measures such as, height, weight and WC. Using data from the CARDIA, this study examines the association between baseline, and longitudinal changes in premenopausal anthropometrics (weight, BMI and WC) and ANM.

2.2 Methods

Study Design, Sample and Data Collection

The CARDIA study is a multicenter longitudinal study of 5,114 (including 2,786 women; one person revoked consent over the course of the study) adults aged 18 to 30 years, who were recruited at baseline (Year 0) in 1985-1986 from four US urban centers (Birmingham, Alabama; Chicago, Illinois; Minneapolis, Minnesota; and Oakland, California) to examine cardiovascular risk factor trends in young adults. The response rate among individuals invited to participate was 50%. This sample has been followed for the past 25 years, with follow-up examinations occurring during 1987–1988 (Year 2), 1990–1991 (Year 5), 1992–1993 (Year 7), 1995–1996 (Year 10), 2000–2001 (Year 15), 2005–2006 (Year 20), and 2010–2011 (Year 25). A majority of the group has been examined at each of the follow-up examinations (91%, 86%, 81%, 79%, 74%, 72%, and 72%, respectively). Approximately 88% of participants have attended ≥4 CARDIA examinations. For the current investigation, data from all exam cycles (i.e., Years 0, 2, 5, 7, 10, 15, 20, and 25) were included in the analyses. The sample was recruited to be balanced on sex, race (White or Black), age (18-24 or 25-30 years), and education (≤12, >12 years). Data collection and follow-up protocols were approved by the institutional review boards of each field center with all participants providing written informed consent. Details of the study design and methods are described elsewhere[20]. Women who did not attend the year 25 visit were more likely to be Black, younger, and current smokers, and to have fewer years of education at baseline[20]. At every CARDIA examination, standardized protocols were used to collect information on demographics, anthropometrics, lifestyle and behavioural factors, medical history, biomarkers, and medication use from participants. The study was approved by the institutional review boards from each field center and the coordinating center. Access to the CARDIA database with information from baseline to Year 25 was obtained from the National Heart, Lung and Blood Institute (NHLBI) for this analysis. Ethics approval was obtained for secondary data analysis for the present study from the York University Research Ethics Board.

Sample Selection Criteria

A woman's anthropometric data from a particular exam were excluded if she was pregnant at that exam, although her other exam data were retained in the analyses. Women who had undergone menopause before 40 years of age (n=17) were excluded from this study, in order

to exclude women with premature ovarian insufficiency, which is of a different etiology than natural menopause. Women diagnosed with breast, ovarian, or endometrial cancers (n=19) were also excluded since treatment for these cancers includes hormone use which might mask the true age of menopause[3]. Also excluded were women who were missing data at follow up by Year 15, when menopause was first ascertained (n=558). Therefore, this analytic sample included 2192 unique women with complete information on the outcome and exposures variables. Participants excluded from the analytic sample were more likely to be Black, and younger (by design) [20]. Missing data on either weight, BMI or WC among women who completed the different cycles, ranged between 1-7%. Those with missing information on exposure variables at each cycle differed with respect to age, race, and education, and parity, presence of diabetes, hypertension, high cholesterol, and menopause status than those with complete information on those variables.

Outcome variable

Age at natural menopause is defined as the age of last menses, occurring after at least 12 months of amenorrhea as per the WHO's definition[21]. Age at natural menopause (ANM) was the outcome for this analysis. Menopausal status, captured at Year 15 (women were 33 to 45 years old), at year 20 (women were 38-50 years old), and at Year 25 (women were 43 to 55 years old) exams, was assessed with the question "Have you gone through menopause or the change of life?" followed by "If yes, how did your periods stop?" with the options of "naturally", "surgically" or "other". Participants were then asked "How old were you when this occurred?" which was used in this analysis as age at menopause. Among postmenopausal women, those who reported cessation of menstrual bleeding not preceded by hysterectomy, radiation, or chemotherapy were classified as having a natural menopause, whereas those who reported menstrual cessation due to hysterectomy and/or bilateral oophorectomy were classified as having surgical menopause. Age at menopause was defined as self-reported age at natural cessation of menstrual flow or age at surgery to remove the uterus and/or ovaries. Women were additionally asked about ever use of oral contraceptives or current hormone therapy.

Independent variables

Anthropometric variables which comprise baseline weight, BMI, and WC, as well as changes in weight, BMI and WC, were the primary time-varying independent variables.

Anthropometrics were measured at each examination. Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared. Body weight was measured on a calibrated balance scale while participants were dressed in light clothing and without shoes by trained and certified technicians using a calibrated scale. Height (without shoes) was assessed by using a vertical ruler and recorded to the nearest 0.5 cm. WC was measured at the minimum abdominal girth (to the nearest 0.5 cm). Due to its stronger correlation with visceral and abdominal obesity than waist to hip ratio (WHR) [18], WC was considered in the analysis rather than WHR. BMI categories include underweight (BMI<18.5 kg/m^2), normal weight (BMI=18.5-24.9 kg/m^2), overweight (BMI=25-29.9 kg/m^2) and obese (BMI≥30 kg/m^2)[22]. Absolute changes in weight, BMI and WC were calculated by subtraction of the baseline measurement from each follow-up measurement. Absolute changes in weight, WC and BMI from the immediate prior visit were also obtained to assess for the role of recent changes in body size. Relative changes in BMI, weight and WC were quantified as the percent changes from baseline and the immediately prior visits. Changes in weight, WC and BMI were made categorical in order to make clinical recommendations easier to interpret, with the categories previously used by Gold et al [18].

Covariates

Time-varying covariates, measured at each exam, included sociodemographic factors, such as education (high school or less, more than high school), current employment (yes/no), and household income in USD (<25,000, 25,000-49,999, 50,000-99,999, ≥100,000); Health behavior variables that included smoking status (never, former, or current), alcohol consumption in the past year (yes/no), ever oral contraceptive (OC) use (yes/no), and self-reported physical activity intensity (vigorous/ moderate/ low). Cigarette smoking was assessed by means of an interviewer-administered tobacco questionnaire. Physical activity level was assessed using a modified version of the Minnesota Leisure Time Physical Activity Questionnaire, with total scores representing self-reported participation in 13 different categories of exercise over the past year, expressed in exercise units[23]. As a useful reference, 200 exercise units (EU) are roughly equivalent to regularly engaging in exercise at 6 METS (moderate intensity), such as stationary bicycling or swimming 2 h a week for 11 months per year. Vigorous physical activity intensity was defined as > 200 exercise units, 100- 200 exercise units corresponded to moderate intensity physical activity, while low intensity physical activity was defined as <100 exercise units.

Alcohol intake was defined as consumption of any alcoholic beverages in the past year. Clinical factors that included physical measurements (e.g. blood pressure), CVD markers, such as total cholesterol level, and parity (none versus 1 or more) were also considered as time varying covariates. At baseline, blood pressure was measured using a random-zero sphygmomanometer with participants seated and after 5 minutes of rest. At the 20-year follow-up examination, three measurements of seated blood pressure were taken from the right arm of each participant after resting for 5 minutes using an Omron HEM907XL automated BP monitor using an appropriately sized cuff and calibrated to the random-zero measures[24]. The average of the second and third consecutive measurements was used for analysis. Hypertension was defined as self-reported diagnosis of hypertension, or SBP>140mm Hg or DBP>90mm Hg or use of anti-hypertensive medications. Before each CARDIA exam, participants fasted for ≥ 8 h and were asked to avoid smoking and heavy physical activity for the final 2 h. Blood was drawn by venipuncture according to a standard protocol[20]. Fasting glucose was measured using hexokinase coupled to glucose-6-phosphate dehydrogenase at a collaborative studies clinical laboratory (Minneapolis, MN) [25]. Type 2 diabetes was defined as a self-reported diagnosis of diabetes, a fasting glucose level of at least 126 mg/dL (to convert to mmol/L, multiply by 0.0555), or reported use of diabetes medications. Blood samples were collected at each clinical center according to a common protocol. Fasting plasma total cholesterol, triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were measured enzymatically by Northwest Lipid Research Laboratories (Seattle, Washington, D.C.)[26]. Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald equation[26]. Hyperlipidemia was defined as self-reported diagnosis of high cholesterol, a total cholesterol level of 240 mg/dL or greater (to convert to mmol/L, multiply by 0.0259), or use of high cholesterol medications. Details of quality control activities that were conducted at CARDIA field centers, coordinating centers, laboratories, and reading centers are available on the CARDIA website at www.cardia.dopm.uab.edu. For covariates with missing data (all<5%), missing values were assigned to a missing indicator category, and analyses using missing indicator categories were conducted to maximize statistical power. Results from analyses restricted to women with complete covariate data were comparable. For the purpose of this analysis, time invariant covariates, include race measured at baseline, parity measured at Year 5, and intake of protein, fat and carbohydrates (CHO). A quantitative food frequency (CARDIA Dietary History) was administered by a trained

interviewer at assess intake of protein, fats, and CHO, at baseline and Year 7. Intake of each food group was calculated as the sum of the number of times a food in each food group was eaten per day (g/d). The CARDIA dietary history is a reasonably reliable and valid (r=0.5-0.7; the CARDIA food questionnaire versus food diaries obtained from a subsample) dietary survey method for obtaining information about habitual intakes in both races[22-25]. The analyses were conducted using Year 7 dietary data, because the data were based on an updated and more extensive version of the food database[22-25]. The natural log of fasting insulin and dietary intake variables was taken since these variables were not normally distributed.

Statistical Analysis

Baseline characteristics of the women were compared by menopausal status using chi-square tests for categorical variables and Kruskal-Wallis tests for continuous variables. Due to the interrelationship between the main independent variables, namely weight, BMI and WC that constitute body size, baseline values and the change in these main independent variables were examined in separate models. Next, discrete–time survival analysis were used to determine the association between anthropometric measures at baseline, absolute and relative changes in those measures and ANM while adjusting for the covariates listed above, including time-varying covariates. Hazard ratios (HRs) and their 95% confidence intervals (CIs) for earlier or later ANM were then estimated by a series of separate Cox proportional hazards models. Women who did not experience menopause were considered as censored observations. Age, rather than time until incident menopause, was used as the time scale for survival analysis in order to address left-truncation, the time at which participants contributed unobserved time at risk. The endpoint age was defined as one of the following: a) age at natural menopause for postmenopausal women (n=316), b) age at last follow up exam if a woman is premenopausal (n=1,372), c) age at hysterectomy or bilateral oophorectomy before having ≥12 consecutive months of untreated amenorrhea (n=324), and d) age at HT initiation (among women who had used HT prior to menopause) (n=18). Criteria for censored observations are similar to that used by Gold et al[14]. To assess for other potential confounders, non-intentional weight change due to non-gynecological cancers or endocrine disrupting conditions, ulcerative colitis, Crohn's disease, rheumatoid arthritis or systemic lupus throughout the study duration was determined. None of the women included in this analysis had developed these conditions. Women who underwent a

hysterectomy after menopause were not censored. Multivariate regression models included covariates selected *a priori* as factors associated with age at natural menopause and/or body size in the literature. Model 1 controlled for the following variables: education level, current employment, household income, OC use, smoking status, alcohol intake in the last year, physical activity intensity, and presence of type 2 diabetes, hypertension, and hyperlipidemia, dietary fat, CHO and protein intake, and parity, as well as fasting glucose, insulin, total cholesterol, LDL-C, HDL-C, TG, Systolic and Diastolic BP. Model 2 was identical to model 1 but excluded type 2 diabetes, hypertension and hyperlipidemia. The reason for creating two distinct models was to address collinearity between metabolic and chronic conditions variables, especially when variance inflation factor (VIF) values were more than 2.5 for some metabolic markers. The assumption of proportional hazards was checked both by inspection plots and Schoenfeld residuals, which did not indicate violation of the assumption (Appendix A). Goodness of fit of the multivariate Cox models was assessed using the likelihood ratio test and Cox-Snell residuals, which indicated a good overall fit (Appendix A). Statistical analysis was performed using STATA software, version 13.0. Statistical significance was set at $\alpha = 0.05$. To minimize multiple comparison bias, a *post hoc* Bonferroni correction was applied, with the uncorrected confidence intervals presented here being similar to those with correction.

2.3 Results

Table 1 shows the baseline characteristics by the menstrual status. The majority of women were still premenopausal (67.3%), while 16.5% were naturally menopausal. The median ANM among women in the CARDIA was 52 years (Interquartile range: 50-54). Blacks were more likely to be pre-menopausal, or were more likely to use HT before menopause than Whites. Higher education level was associated with being premenopausal. Women who had a higher household income (50,000-99,999 USD), who were employed, who were current smokers, were more likely to be premenopausal or to be using HT before menopause. Women who were diagnosed with diabetes, hypertension or hyperlipidemia, were more likely to be pre-menopausal. The majority of women had gained >10% in weight (29.8%), BMI (28.9%) and WC (27%) since baseline, as Table 2 shows. Women were more likely to have gained 1-5% in weight (13.6%), BMI (12.9%), and WC (14.9%), respectively, from one visit to the next.

Results of the adjusted Cox regression models with weight, BMI and WC as time-varying variables are outlined in Tables 3a, 3b and 3c. Earlier ANM was strongly associated with current smoking in both models. The following variables were also significantly related to an earlier ANM in both models and across anthropometric measures: past smoking, fat intake change and changes in diastolic and systolic blood pressure. Pre-menopausal hypertension was strongly associated with an earlier ANM in the model for weight (HR=1.32; 95% CI=1.04-1.66), BMI (HR=1.32; 95% CI=1.05-1.68), and WC (HR=1.35; 95% CI=1.05-1.68), respectively. Baseline WC (HR=0.98; 95% CI=0.97-0.99) was significantly related to a later ANM in models 1 and 2. A higher education level was also associated with later ANM in all anthropometric measures. Baseline weight, BMI, and changes in anthropometric measures were not associated with ANM.

Multivariate Cox regression of the study participants' changes in anthropometrics as a categorical variable, showed that if there was >5% weight loss since baseline, the hazard rate for an earlier ANM decreased by 45% (HR=0.55, 95% CI=0.31-0.97). This association was no longer statistically significant when hypertension, diabetes and hyperlipidemia were removed from the analysis, as shown in Table 4.

2.4 Discussion

The aim of this study was to assess whether baseline and longitudinal changes in adiposity are associated with ANM. No association between changes in anthropometrics across time and onset of menopause was observed; but, baseline WC was negatively associated with ANM. Moreover, the presence of hypertension prior to menopause was also associated with an earlier ANM. It was found that women who lost more than 5% of their weight since baseline measurement did have a later menopause than women with stable weight, after controlling for potential confounding variables. Findings might inform prevention strategies that benefit many women going through the menopausal transition, highlighting the importance of both control and prevention of cardiovascular risk factors, especially blood pressure, in adulthood prior to menopause when cardiovascular risk factors are prone to change.

The lack of association found between any changes in anthropometrics throughout the study period was similar to Hardy et al[13], who using a nationally representative British cohort of 1583 women born in March 1946 reported no relationship between a BMI trajectory from 20 to 36 years and ANM. Similarly, Gold et al[18], using data from the SWAN, showed that percentage weight change since baseline and since prior visit were unrelated to any incident vasomotor symptoms (VMS), a proxy to menopause onset, in either early or late menopause. This is also in concordance with Galicchio et al[34] who in a longitudinal study of midlife women demonstrated that change in BMI and weight over 1-5 years of follow up was not linked to hot flashes in two racial groups. In contrast, women who reported severe weight cycling i.e. women who lost ≥20 pounds three or more times between ages 18 and 35, had higher odds (OR=1.48, 95%CI=1.04, 2.10) of early natural menopause compared to normal weight women, as data from the Nurses' Health Study 2 (NHS2), revealed[19]. Weight change in that study was calculated by subtracting weight at age 18 from that at age 35. The study's assessment of longitudinal changes of adiposity measures was limited to premenopausal women until age 35 with weight loss or gain being self – reported. Morris et al[16] observed that a greater weight gain from 20 to 40 years of age was associated with a later menopause (HR=0.93, 95%CI=0.87, 0.98) after adjustment for BMI at age 40 years and other factors.

Although several studies indicate that overweight decelerates menopause, others note no effect of body weight menopause onset[3, 4, 13, 14, 27, 28]. Of those that found an association, large weight gain from age 20 years[3] was related to a later menopause and large weight losses to an earlier menopause[12]. Our conflicting result that women with weight loss of more than 5% since baseline had a later menopause might be due to residual confounding by age or other factors such as endogenous hormone levels[15] (e.g. follicular stimulating hormone and estradiol). Our result was similar to that of Aydin [15] who, in a cross-sectional study, conducted among postmenopausal Turkish women, found that weight loss of more than 5 kg was involved in postponing menopause. In fact, women with low BMI have high levels of Anti-Mullerian Hormone (AMH) [19], a marker of ovarian reserve related to antral follicle count that is produced by the granulosa cells in the ovary, indicating a low risk for earlier menopause. Over a quarter of women in our sample who had lost more than 5% of their weight were overweight at baseline (data not shown), and so they may be more likely to intentionally lose weight throughout the years. Although we were able to adjust for several potential confounding factors, confounding by unknown or unmeasured covariates, such as hormone levels and changes in dietary habits and physical activity, cannot be ruled out. Therefore, this result might be spurious. Aging also results in a decrease in lean body mass, which decreases resting metabolic rate[31]. Although estrogen deprivation after menopause leads to an increase in total body fat, it also results in a decrease in lean body mass, such that there is little net effect on weight related to menopause alone[32]. It might be that women who lost more than 5% of their weight over 25 years of follow up have lost muscle more than fat. Additional studies should assess longitudinal relationships between birth weight, early life adiposity, and ANM.

Consistent with previous research, another finding from our analysis was that premenopausal hypertension was strongly associated with an earlier menopause among women in the CARDIA. Analysis from the Korea National Health and Nutrition Examination Survey (KNHANES)[36], based on 13,584 women, also noted that hypertension diagnosed before age 40 was associated with an earlier ANM (HR=1.03, 95% CI=1.02-1.03). In fact, middle-aged women require careful monitoring during the menopausal transition. For example, a study also using the CARDIA by Ebong et al[38] documented an association between high sensitivity C - reactive protein (hs-CRP), an inflammatory biomarker, and hypertension among premenopausal women,

independent of BMI. Estrogen is thought to protect against CVD in premenopausal women via both systemic and local vascular effects. These vasoprotective effects are mediated in part through the binding of estrogen to vascular endothelial cells and smooth muscle cells, affecting their function and resulting in decreased vasoconstriction and blood pressure[39, 40]. Weight, cholesterol level, diabetes mellitus, blood pressure, and smoking have negative vascular effects, but may also affect hormone production, which in turn could have an impact on menopausal age by altering estrogen levels. Therefore, the interplay and overlap between the effects of inflammation, obesity and estrogen loss may contribute to the development of hypertension in the postmenopausal period. Our results showed that systolic blood pressure and diastolic blood pressure were both associated with age at menopause, which is in concordance with results from the Framingham Heart study[17]. Data from Janssen et al[41] also found that progression through menopause was associated with an increase in the incidence of the metabolic syndrome (MetS) where the odds of developing MetS were 1.45 (95% CI=1.35-1.56) per year during the perimenopause and 1.24 (95% CI=1.18-1.30) per year in the postmenopausal years.

Our results showed that any change in dietary fat intake per day was associated with an earlier ANM. The few prospective cohort and cross-sectional studies that have investigated the effect of dietary fat on menopause timing have had inconsistent results[7, 42-46]. A German cohort study reported that higher total intakes of fat, meat and protein were all correlated with later onset of menopause[44]. On the contrary, another study using NHS2 data[45] found that only a daily intake vegetable-based protein was associated with a later ANM. Further longitudinal research is needed to elucidate the relation of dietary factors with ANM. In addition, factors consistently related with age at menopause such as higher education levels, and current smoking status were significantly associated with ANM as indicated in other studies[3-7, 14].

Baseline WC was a consistent, yet modest predictor of later ANM in both models, as per the results of the multivariate Cox regression. A possible explanation for this result might lie in the fact that WC is a better predictor of and correlated with cardiovascular risk factors such as hypertension, dyslipidemia, and MetS than BMI and weight [47]. Vice versa, SBP, DBP, cholesterol levels, glucose levels, and diabetes are also independent predictors of WC [47, 48]. Moreover, a *post hoc* regression analysis of our data also revealed that hypertension, diabetes

and insulin levels were prominent predictors of WC. Baseline body size measures are highly correlated with current body size[35, 36], therefore our result might be in line with the notion that premenopausal obesity delays menopause. Our results were similar to Gold et al[18] who noted that higher WC was significantly associated with lower VMS risk in late menopause. It should be noted that while body size indicators often increase with age, the current analysis could not distinguish between period effects and life transitions.

Strengths and Limitations

The strengths of the present study include the detailed exposure and outcome information available. This is the first study to evaluate associations of three dynamic body size indicators as risk factors of ANM. CARDIA's design provides a clear temporality between exposure and outcome, and thus builds upon previous literature. Furthermore, the adjustment of multiple established risk factors and censoring at age of initiation of hormone therapy and age at surgical menopause limits the potential for confounding bias. All anthropometrics were objectively assessed and obtained across all cycles, therefore allowing examination of body size prior to menopause and avoiding the potential for recall or reporting bias observed in previous studies. Indeed, few previous studies have considered BMI or weight in earlier adult life or change in these measures, and most of those that have were limited by recall bias resulting from the use of retrospective self-reported measures of earlier weight[3, 12,13,16,19]. The bi-racial nature of the cohort also enabled examining associations across racial groups. Moreover, many of the potential confounding variables were considered to be time-variant so their associations with ANM can be taken as meaningful.

Nonetheless, a few caveats should be mentioned. First, generalizability might be limited due to the self-selected nature of the sample. Recall bias due to self-report of ANM might be another limitation; however, reporting of menopause has been found to be highly reproducible[14, 50]. The data were subject to right censoring because participants had "survived" (not yet postmenopausal). A large proportion of women in the youngest age groups had not yet reached menopause, but the analysis methods, which are standard for this type of study[4, 14], allowed for their inclusion. Our data were also left-truncated since women with a reported age at menopause below age 40 were not present in the sample. However, our analyses accounted for entry at ages 40 years and above. Lastly, some of our results might be due to chance given the number of

multiple comparisons; however, this has been minimized after performing a *post hoc* Bonferroni correction and comparing the corrected and uncorrected confidence intervals, which were similar.

2.5 Conclusion

This study is one of the few prospective studies of objectively assessed anthropometric measures and ANM. Taken together, these findings suggest that baseline WC, and more than a 5 % loss of baseline weight are modest predictors of ANM in middle-aged women. Modest effect sizes for the relationship between longitudinal changes in body size indicators and ANM were obtained, yet they are of clinical significance since results were consistent with previous research. Premenopausal hypertension and blood pressure indicators were also significantly related to an earlier ANM, highlighting that menopausal age is at least in part determined by modifiable factors. Further research is warranted on the role of longitudinal changes in adiposity and ANM. A better understanding of how weight gain or loss affects menopause onset and ovarian aging may help inform women and their health care providers of ways to modify their risk of an earlier menopause and the health conditions associated with it.

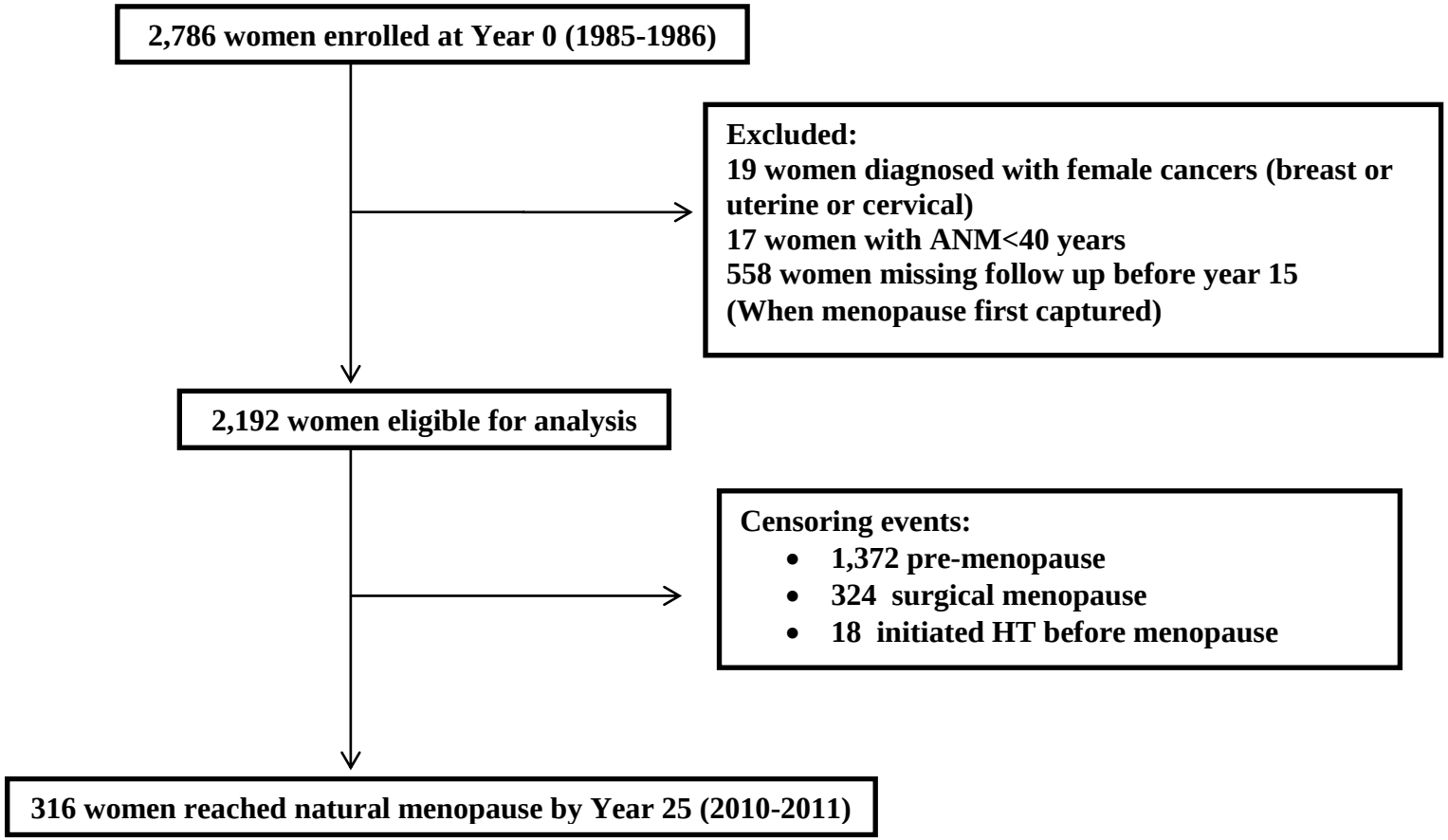

Figure 2.1: Flowchart of participants in the CARDIA study

Table 2.1: Baseline sociodemographic, health behaviour, and clinical characteristics of women in the study sample by menstrual status, CARDIA (1985-2011).

	Total	Pre-menopause (n=1,372)	Natural Menopause (n=316)	Surgical Menopause (n=324)	HT use prior to natural menopause (n=18)
Characteristics					
Age , years, (mean, SD)	24.8(3.65)	24.0(3.59)	27.9(2.28)	26.25(3.12)	28.5(1.92)
Race (n, %)					
White	1,204 (47.0)	709(70.7)	205(20.4)	89(8.9)	3(6.82)
Black	1,357(53.0)	663(65.7)	111(11.0)	235(23.3)	14(25.5)
Educational level (n, %)					
High school graduate or less	966(35.2)	480(67.6)	84(11.8)	146(20.6)	3(10.3)
More than High school	1,780(64.8)	892(68.5)	232(17.8)	178(13.7)	14(20.0)
Income, USD* (n, %)					
<25,000	748(38.9)	427(67.7)	95(15.1)	109(17.3)	4(16.0)
25,000-49,999	704(36.7)	430(71.3)	81(13.4)	92(15.3)	4(14.3)
50,000-99,999	273(14.2)	161(66.3)	52(21.4)	30(12.4)	7(30.4)
≥ 100,000	--	--	--	--	--
Currently Employed (n, %)	1,666(65.1)	888(66.5)	236(17.7)	212(15.9)	16(20.5)
Smoking Status					
Never	1,468(57.5)	846(71.0)	161(13.5)	184(15.5)	8(15.1)
Past	349(13.7)	196(67.4)	55(18.9)	40(13.8)	4(25.0)
Current	736(28.8)	327(62.5)	97(18.6)	99(18.9)	4(13.8)
Alcohol intake in the last year (n, %)	2,145(83.6)	1,161(68.5)	277(16.3)	257(15.2)	16(18.4)
Oral contraceptive use (n, %)	814(41.7)	473 (74.5)	75(11.8)	87(13.7)	5(18.5)
Physical activity intensity, exercise units, (mean, SD)	335(249.7)	354(260)	362(240)	284(241)	459.3(296)
Parity (n, %)					
1 or more	2,577(93.9)	1,272(68.4)	292(15.7)	296(15.9)	16(17.0)
Weight, Kg, (mean, SD)	72.5(19.0)	72.1(19.4)	70.8(17.2)	75.8(18.7)	69.3(13.6)
Waist circumference (WC), cm, (mean, SD)	74.0(11.0)	73.70(11.00)	73.2(10.2)	76.1(10.9)	70.2(7.30)
BMI, Kg/m^2 (mean, SD)	24.5(5.52)	24.3(5.38)	23.9(5.15)	25.5(5.64)	22.9(3.64)
Dietary fat, g/day, (mean, SD)[a]	98.9(71.5)	95.93(70.8)	94.5(61.4)	102(56.9)	67.7(21.4)
Dietary CHO, g/day, (mean, SD)[a]	216(131.6)	214(129.5)	205(115.6)	217(119.6)	166.9(70.8)
Dietary protein, g/day, (mean, SD)[a]	76.6(53.6)	75.0(50.12)	77.4(55.8)	74.5(38.2)	62.8(19.0)
Fasting glucose, mg/dl, (mean, SD)	80.8(16.8)	80.2(13.24)	81.5(15.7)	81.5(15.3)	79(12.3)
Fasting insulin, mg/dl, (mean, SD)	11.4(8.34)	11.16(8.10)	9.4(6.03)	12.5(9.7)	7.6(2.80)
HDL cholesterol, mmol/L,(mean, SD)	55.3(12.8)	55.0(12.8)	58.4(12.7)	54.8(12.6)	66.3(8.40)
LDL cholesterol, mmol/L,(mean, SD)	108(30.2)	109(30.2)	105(31.4)	110(29.3)	99.5(27.5)
Triglycerides, mmol/L, (mean, SD)	66.1(35.0)	66.9(36.9)	62.0(30.1)	68.7(34.2)	53.3(22.2)
Total cholesterol, mmol/L, (mean, SD)	177(32.7)	178(32.5)	175(32.0)	179(30.5)	170.3(26.3)
Systolic blood pressure, mm Hg , (mean, SD)	106(9.62)	106(9.22)	104(9.12)	107(9.67)	100.8(8.52)
Diastolic blood pressure, mm Hg , (mean, SD)	66.7(8.90)	67.0(8.53)	65.2(8.60)	67.6(8.80)	62.7(9.00)
Current Diabetes (n, %)	261(9.50)	29(51.8)	6(10.7)	21(37.5)	0
Current Hypertension (n, %)	403(14.7)	108(65.5)	19(11.5)	38(23.0)	0
Current Hyperlipidemia (n, %)	355(12.9)	83(65.4)	23(18.1)	21(16.5)	0

[a] Information not available at year 0; year at which characteristic was first assessed was taken as baseline.

*Chi-square test for categorical variables; Kruskal-Wallis test for continuous variables; majority had *p*-values of <0.001.

Note: percentages in the last four columns are row %, however only the %'s within the first three of those columns add to a 100%, since HT use prior to menopause was a variable on its own (yes/no).

Table 2.2: Total percent distribution of relative longitudinal changes in anthropometric measures among women in the CARDIA, (1985-2011).

	Weight	**BMI**	**WC**
Relative change since baseline, per 1%	**%**	**%**	**%**
Lost >5%	4.25	4.61	3.30
Lost 1%-5%	4.73	5.06	5.42
Stable	14.9	14.7	14.9
Gained 1%-5%	8.31	8.37	9.77
Gained>5%-10%	9.89	9.92	11.1
Gained>10%	29.8	28.9	27.0
Relative change since prior visit, per 1%			
Lost >5%	6.01	6.19	5.12
Lost 1%-5%	7.60	8.00	8.68
Stable	6.43	6.32	6.59
Gained 1%-5%	13.6	12.9	14.9
Gained>5%-10%	11.5	11.4	11.4
Gained>10%	9.33	9.44	7.33

Table 2.3a: Adjusted Hazard Ratios (HRs) for the association between weight and ANM in the CARDIA, (1985-2011).

	Model 1*		Model 2[¥]	
	HR	**95%CI**	**HR**	**95%CI**
Baseline Weight	0.99	0.99-1.02	0.99	0.99-1.01
Time-varying Weight	1.00	0.99-1.00	1.00	0.99-1.01
Race				
Black	0.92	0.74-1.15	0.92	0.74-1.15
Educational level				
High school graduate or less	1.00		1.00	
More than High school	**0.77**	**0.62-0.97[a]**	**0.75**	**0.60-0.94 [a]**
Household income, USD				
<25,000	1.00		1.00	
25,000-49,999	1.09	0.83-1.44	1.06	0.84-1.32
50,000-99,999	0.95	0.70-1.31	0.92	0.71-1.21
≥100,000	1.09	0.72-1.65	1.07	0.73-1.56
Currently Employed	0.95	0.80-1.13	0.95	0.80-1.13
Smoking Status				
Never	1.00		1.00	
Past	**1.24**	**1.03-1.50[a]**	**1.25**	**1.03-1.51[a]**
Current	**1.87**	**1.46-2.40[b]**	**1.86**	**1.45-2.40[b]**
Alcohol intake in the last year	1.11	0.86-1.41	1.12	0.87-1.42
Oral Contraceptive use	0.82	0.66-1.03	0.91	0.75-1.11
Physical Activity				
Low Intensity	1.00		1.00	
Moderate Intensity	0.98	0.75-1.29	0.95	0.72-1.25
High Intensity	1.09	0.86-1.38	1.11	0.87-1.40
Parity				
1 or more	1.17	0.88-1.56	1.17	0.88-1.56
Diabetes	0.74	0.47-1.16	--	--
Hypertension	**1.32**	**1.04-1.66[a]**	--	--
Hyperlipidemia	0.96	0.74-1.22	--	--
Log Dietary fat, g/day	**1.37**	**1.01-1.84[a]**	1.35	1.00-1.81
Log Dietary CHO, g/day	0.83	0.61-1.12	0.83	0.61-1.12
Log Dietary protein, g/day	1.01	0.69-1.49	1.02	0.69-1.50
Fasting glucose, mg/dl,	0.99	0.99-1.01	0.99	0.99-1.01
Log Fasting insulin, mg/dl,	1.01	0.99-1.02	0.94	0.76-1.15
HDL cholesterol, mmol/L	1.05	0.86-1.29	1.06	0.87-1.30
LDL cholesterol, mmol/L	1.05	0.85-1.28	1.05	0.86-1.29
Triglycerides, mmol/L,	1.01	0.97-1.05	1.01	0.97-1.05
Total cholesterol, mmol/L,	0.96	0.78-1.17	0.95	0.78-1.17
Systolic blood pressure, mm Hg	**0.98**	**0.97-0.99[b]**	**0.98**	**0.98-0.99[b]**
Diastolic blood pressure, mm Hg	**1.02**	**1.01-1.03[b]**	**1.02**	**1.01-1.04[b]**

*Model 1: Adjusted for the following covariates in addition to baseline values for weight: Time-varying covariates include education, current employment, household income, OC use, current smoking, alcohol intake in the last year, physical activity intensity, and presence of diabetes, hypertension and hyperlipidemia, in addition to Fasting glucose, insulin, LDL-C, HDL-C, TG, Total cholesterol, Systolic and Diastolic BP. Time invariant covariates include race, dietary fat, CHO and protein intake and parity.

¥ Model 2 was adjusted to include all variables in model 1 except: diabetes, hypertension and hyperlipidemia.

[a] p- value≤0.05; [b] p-value≤0.0001

Table 2.3b: Adjusted Hazard Ratios (HRs) for the association between BMI and ANM in the CARDIA, (1985-2011).

	Model 1*		**Model 2¥**	
	HR	**95%CI**	**HR**	**95%CI**
Baseline BMI, kg/m^2	0.98	0.95-1.01	0.97	0.94-1.00
Time-varying BMI	0.99	0.98-1.02	1.01	0.98-1.03
Race				
Black	0.94	0.75-1.17	0.94	0.75-1.17
Educational level				
High school graduate or less	1.00		1.00	
More than High school	**0.77**	**0.62-0.96[a]**	**0.75**	**0.60-0.94[a]**
Household income, USD				
<25,000	1.00		1.00	
25,000-49,999	1.08	0.82-1.43	1.05	0.84-1.31
50,000-99,999	0.94	0.68-1.29	0.91	0.70-1.19
≥100,000	1.08	0.71-1.63	1.06	0.72-1.54
Currently Employed	0.96	0.81-1.15	0.96	0.81-1.15
Smoking Status				
Never	1.00		1.00	
Past	**1.25**	**1.03-1.52[a]**	**1.26**	**1.04-1.52[a]**
Current	**1.89**	**1.47-2.41[b]**	**1.88**	**1.47-2.42[b]**
Alcohol intake in the last year	1.10	0.86-1.40	1.11	0.87-1.41
Oral Contraceptive use	0.82	0.65-1.03	0.91	0.75-1.12
Physical Activity				
Low Intensity	1.00		1.00	
Moderate Intensity	0.99	0.75-1.31	0.96	0.73-1.26
High Intensity	1.08	0.86-1.40	1.10	0.87-1.39
Parity				
1 or more	1.16	0.87-1.55	1.16	0.87-1.55
Diabetes	0.75	0.48-1.19	--	--
Hypertension	**1.32**	**1.05-1.68[a]**	--	--
Hyperlipidemia	0.95	0.74-1.21	--	--
Log Dietary fat, g/day	**1.36**	**1.01-1.84[a]**	1.35	1.00-1.81
Log Dietary CHO, g/day	0.83	0.61-1.12	0.83	0.61-1.12
Log Dietary protein, g/day	1.02	0.70-1.50	1.03	0.70-1.51
Fasting glucose, mg/dl,	1.01	0.99- 1.01	0.99	0.99-1.01
Log Fasting insulin, mg/dl,	1.01	0.99- 1.02	0.96	0.78-1.18
HDL cholesterol, mmol/L	1.06	0.86- 1.30	1.07	0.87-1.30
LDL cholesterol, mmol/L	1.05	0.86- 1.29	1.06	0.86-1.29
Triglycerides, mmol/L,	1.01	0.97- 1.05	1.01	0.97-1.05
Total cholesterol, mmol/L,	0.95	0.78- 1.16	0.95	0.77-1.16
Systolic blood pressure, mm Hg	**0.98**	**0.98-0.99[a]**	**0.98**	**0.98-0.99[a]**
Diastolic blood pressure, mm Hg	**1.02**	**1.01-1.03[a]**	**1.02**	**1.01-1.04[a]**

*Model 1: Adjusted for the following covariates in addition to baseline values for BMI: Time-varying covariates include education, current employment, household income, OC use, current smoking, alcohol intake in the last year, physical activity intensity, and presence of diabetes, hypertension and hyperlipidemia, in addition to Fasting glucose, insulin, LDL-C, HDL-C, TG, Total cholesterol, Systolic and Diastolic BP. Time invariant covariates include race, dietary fat, CHO and protein intake and parity.

¥ Model 2 was adjusted to include all variables in model 1 except: diabetes, hypertension and hyperlipidemia.

[a] p- value<0.05; [b] p-value<0.0001

Table 2.3c: Adjusted Hazard Ratios (HRs) for the association between waist circumference and ANM in the CARDIA, (1985-2011).

	Model 1*		Model 2¥	
	HR	**95%CI**	**HR**	**95%CI**
Baseline WC, cm	**0.98**	**0.97-0.99[a]**	**0.98**	**0.97-0.99[a]**
Time-varying WC	1.01	0.99-1.02	1.01	0.99-1.02
Race				
Black	0.91	0.72-1.13	0.91	0.73-1.13
Educational level				
High school graduate or less	1.00		1.00	
More than High school	**0.77**	**0.61-0.95[a]**	**0.74**	**0.59 -0.92[a]**
Household income, USD				
<25,000	1.00		1.00	
25,000-49,999	1.06	0.80-1.40	1.04	0.83-1.30
50,000-99,999	0.91	0.66-1.25	0.89	0.68-1.16
≥100,000	1.08	0.71-1.62	1.06	0.73-1.55
Currently Employed	0.95	0.80-1.13	0.95	0.79-1.13
Smoking Status				
Never	1.00		1.00	
Past	**1.23**	**1.02-1.50[a]**	**1.24**	**1.02-1.50[a]**
Current	**1.89**	**1.47-2.41[b]**	**1.87**	**1.46-2.40[b]**
Alcohol intake in the last year	1.12	0.88-1.44	1.14	0.89-1.45
Oral Contraceptive use	0.81	0.65-1.02	0.89	0.74-1.10
Physical Activity				
Low Intensity	1.00		1.00	
Moderate Intensity	0.97	0.74 -1.28	0.93	0.71-1.23
High Intensity	1.09	0.87-1.30	1.11	0.84-1.40
Parity				
1 or more	1.17	0.88-1.56	1.17	0.88-1.56
Diabetes	0.76	0.48-1.19	--	--
Hypertension	**1.35**	**1.05-1.68[a]**	--	--
Hyperlipidemia	0.96	0.75-1.23	--	--
Log Dietary fat, g/day	**1.38**	**1.03-1.86[a]**	**1.36**	**1.01-1.84[a]**
Log Dietary CHO, g/day	0.81	0.60-1.10	0.82	0.60-1.11
Log Dietary protein, g/day	1.01	0.99-1.01	1.01	0.69-1.49
Fasting glucose, mg/dl,	0.99	0.99-1.01	0.99	0.99-1.01
Log Fasting insulin, mg/dl,	1.01	0.99- 1.02	0.93	0.76-1.15
HDL cholesterol, mmol/L	1.04	0.85- 1.28	1.04	0.85-1.28
LDL cholesterol, mmol/L	1.04	0.85- 1.27	1.04	0.85-1.27
Triglycerides, mmol/L,	1.01	0.97- 1.05	1.01	0.97-1.05
Total cholesterol, mmol/L,	0.97	0.79- 1.18	0.97	0.79-1.18
Systolic blood pressure, mmHg	**0.98**	**0.98- 0.99[a]**	**0.98**	**0.97-0.99[a]**
Diastolic blood pressure, mmHg	**1.02**	**1.01-1.03[a]**	**1.02**	**1.01-1.04[a]**

*Model 1: Adjusted for the following covariates in addition to baseline values for WC: Time-varying covariates include education, current employment, household income, OC use, current smoking, alcohol intake in the last year, physical activity intensity, and presence of diabetes, hypertension and hyperlipidemia, in addition to Fasting glucose, insulin, LDL-C, HDL-C, TG, Total cholesterol, Systolic and Diastolic BP. Time invariant covariates include race, dietary fat, CHO and protein intake and parity.

¥ Model 2 was adjusted to include all variables in model 1 except: diabetes, hypertension and hyperlipidemia.

[a] p- value<0.05; [b] p-value<0.0001

Table 2.4: Hazard Ratios (HR) for the Association between longitudinal changes in anthropometric measures and ANM in the CARDIA, (1985-2011)

	Unadjusted		Model 1*		Model 2¥	
Relative change in weight since baseline, per 1%	**HR**	**95%CI**	**HR**	**95%CI**	**HR**	**95%CI**
Lost >5%	**0.79**	**0.65-0.97***	**0.55**	**0.31-0.97 [a]**	0.51	0.26-1.00
Lost 1%-5%	1.01	0.84-1.20	0.92	0.55-1.54	1.04	0.57-1.91
Stable	1.00		1.00		1.00	
Gained 1%-5%	0.98	0.84-1.14	0.72	0.45-1.15	0.65	0.36-1.15
Gained>5%-10%	0.92	0.80-1.06	0.72	0.46-1.12	0.67	0.39-1.15
Gained>10%	0.99	0.88-1.11	0.72	0.48-1.08	0.67	0.40-1.11
Relative change in weight since prior visit, per 1%						
Lost >5%	1.01	0.83-1.23	1.29	0.90-1.82	1.14	0.74-1.76
Lost 1%-5%	1.01	0.84-1.21	1.05	0.76-1.46	0.90	0.61-1.31
Stable	1.00		1.00		1.00	
Gained 1%-5%	1.05	0.89-1.23	1.15	0.85-1.55	1.11	0.78-1.57
Gained>5%-10%	0.90	0.75-1.07	0.99	0.71-1.38	0.72	0.48-1.09
Gained>10%	1.07	0.89-1.28	1.01	0.71-1.43	0.86	0.56-1.31
Relative change in BMI since baseline, per 1%						
Lost >5%	**0.81**	**0.66-0.99***	0.86	0.48-1.52	0.78	0.39-1.57
Lost 1%-5%	1.01	0.84-1.19	1.26	0.73-2.16	1.41	0.75-2.64
Stable	1.00		1.00		1.00	
Gained 1%-5%	1.03	0.88-1.19	0.95	0.57-1.57	0.92	0.50-1.67
Gained>5%-10%	0.95	0.82-1.10	1.02	0.63-1.66	1.08	0.61-1.91
Gained>10%	1.03	0.92-1.16	0.96	0.61-1.51	0.96	0.56-1.64
Relative change in BMI since prior visit, per 1%						
Lost >5%	1.08	0.88-1.32	1.11	0.71-1.71	1.13	0.73-1.75
Lost 1%-5%	1.07	0.89-1.29	1.06	0.72-1.56	1.05	0.71-1.55
Stable	1.00		1.00		1.00	
Gained 1%-5%	1.08	0.91-1.27	1.15	0.80-1.65	1.18	0.82-1.68
Gained>5%-10%	0.95	0.79-1.13	0.89	0.60-1.31	0.91	0.61-1.34
Gained>10%	1.16	0.96-1.39	0.91	0.60-1.40	0.93	0.61-1.43
Relative change in WC since baseline, per 1%						
Lost >5%	0.95	0.75-1.20	0.49	0.22-1.08	0.46	0.21-1.02
Lost 1%-5%	1.02	0.85-1.22	0.70	0.38-1.26	0.69	0.38-1.26
Stable	1.00		1.00		1.00	
Gained 1%-5%	1.14	0.99-1.31	0.67	0.41-1.09	0.64	0.39-1.03
Gained>5%-10%	1.01	0.87-1.16	0.69	0.43-1.11	0.68	0.42-1.08
Gained>10%	1.11	0.98-1.25	0.74	0.48-1.14	0.74	0.48-1.14
Relative change in WC since prior visit, per 1%						
Lost >5%	1.04	0.84-1.28	0.85	0.55-1.33	0.85	0.54-1.32
Lost 1%-5%	1.01	0.84-1.21	1.02	0.68-1.52	0.99	0.67- 1.48
Stable	1.00		1.00		1.00	
Gained 1%-5%	1.12	0.95-1.32	0.87	0.61-1.24	0.87	0.61-1.24
Gained>5%-10%	1.06	0.89-1.26	0.85	0.57-1.25	0.85	0.57-1.25
Gained>10%	1.14	0.94-1.38	0.86	0.55-1.33	0.86	0.56-1.34

*Model 1: Adjusted for the following covariates in addition to baseline values for each adiposity measure: Time-varying covariates include education, current employment, household income, OC use, current smoking, alcohol intake in the last year, physical activity intensity, and presence of diabetes, hypertension and hyperlipidemia, in addition to Fasting glucose, insulin, LDL-C, HDL-C, TG, Total cholesterol, Systolic and Diastolic BP. Time invariant covariates include race, dietary fat, CHO and protein intake and parity.

¥ Model 2 was adjusted to include all variables in model 1 except: diabetes, hypertension and hyperlipidemia.

[a] p- value≤0.05

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Chapter 3

Age at natural menopause (ANM) and its associated factors in Canada: Cross-sectional Analyses from the Canadian Longitudinal Study on Aging (CLSA)

Abstract

Background: Early onset of menopause is associated with long term disease and higher mortality risks. Research suggests that age at natural menopause (ANM) varies across populations. Little is known about factors that affect ANM in Canadian women.

Objective: This study aims to estimate the median ANM and examine factors associated with earlier ANM among Canadian women.

Methods: Baseline data from the Tracking cohort of the Canadian Longitudinal Study on Aging (CLSA) were used for this analysis. The relation of sociodemographic, lifestyle and health related factors with ANM was examined among 7,719 women aged 40 and above. Nonparametric Kaplan–Meier cumulative survivorship estimates were used to assess the timing of natural menopause. Univariate and multivariate Cox proportional hazard regression models were used to characterize ANM and its association with relevant covariates.

Results: Overall, median ANM was 51 years. Having no partner, low household income and education levels, current and former smoking, and cardiovascular disease (CVD) were all associated with an earlier ANM, while current employment, alcohol consumption, and obesity were associated with later ANM.

Conclusion: These findings provide a national estimate of ANM in Canada. These findings show the importance of lifestyle factors and health conditions in determining menopausal age. These factors might help in risk assessment, prevention and early management of chronic disease risk during the menopausal transition.

Keywords: Menopause, Factors, Smoking, Education, Weight, Canada

3.1 Introduction

While secular changes in the average ANM are not as well documented as those as the age of menarche, there is some evidence to suggest that there have been increases in the average age at menopause in the past century[1,2]. Contemporary industrialized populations demonstrate a later mean age at menopause than non-industrialized populations[3]. The median age of menopause in Western countries is between 48 and 52 years[4]. However, the median age range of menopause in developing countries is between 47 to 49.3 years[5]. A recent meta-analysis of 36 studies across the globe (excluding Canada) on the age of menopause resulted in an overall median age of 48.7 years[6]. Distribution by geographical region demonstrated that ANM was generally lower among African, Latin American, Asian and Middle Eastern countries and was highest in Europe and Australia, followed by the USA[6]. Moreover, ANM also varies by race/ethnicity. In fact, Palmer et al[7] found that African American women have an earlier onset of ANM than Caucasian women. Therefore, natural menopause is a complex biosocial and bio cultural phenomenon with geographic and ethnic variations.

Previous studies have shown that variation in age of menopause is associated with several factors such as genetic, reproductive, demographic and lifestyle behaviors. ANM has been positively associated with maternal age at menopause and twin studies have found a significant heritability of ANM[8]. Earlier age of menopause has also been associated with nulliparity, cigarette smoking, lower socioeconomic status and education levels, unemployment, single marital status, non-oral contraceptive (OC) use, and low body mass index (BMI) as reported in a review on the correlates of ANM[8]. However, the majority of these results remain inconsistent across studies. With the increase in life expectancy, the proportion of postmenopausal life also increases. Thus, the impact of menopause on women's health has become more significant from both clinical and public health perspectives. The age at which menopause occurs is critical for women's health trajectory, as it is an indicator of ovarian function and aging, and portends future health risks. Women with an earlier menopause are at an increased risk of cardiovascular disease (CVD) and osteoporosis, while women with a later menopause are at an increased risk of breast and endometrial cancers[8, 11].

Menopause, one of the major components of women's reproductive lives, is not only understood as being integral to women's overall health and wellbeing, but is increasingly recognized as a sentinel of chronic disease in later life. Factors that influence age at menopause may affect not only timing of cessation of menses but also future cardiovascular and reproductive health outcomes. A definition of the factors related to ANM is warranted as a means for quantifying risk for earlier or later menopause as a number of gaps in our understanding of preventable factors that may influence ANM exist. No study to date has provided a nationally representative estimate of the ANM in Canada and has directly examined the correlates of age at menopause. Therefore, this study aims to investigate the correlates of ANM among a population- based, nationally representative sample of Canadian women.

3.2 Methods

Study Design and Sample

Baseline data from the Tracking cohort (version 3.0) of the Canadian Longitudinal Study on Aging (CLSA) were accessed for secondary analysis. In short, the CLSA is a 20 year prospective study of 50,000 men and women aged between 45 and 85 years at baseline. Launched in 2010, the CLSA aims to provide national-level insight into the behaviors and health of adults ages 45 to 85 years at baseline. The study has two categories of participants: i) the Tracking cohort who were randomly selected within age and sex strata in each province and followed by telephone interview, and; ii) the Comprehensive cohort participants, who had more intensive physical measures as well as questionnaire surveys. The Tracking cohort included n=21,241 (males: 10,406; females: 10,835) non-institutionalized participants distributed across all 10 provinces. Individuals living in long-term care institutions, and having cognitive impairment at the time of recruitment were excluded at baseline. However, those living in households and transitional housing arrangements (e.g., seniors' residences, in which only minimal care is provided) were included. The CLSA study design has been previously described in detail elsewhere[9].

Study Participants

Of the initial overall sample that included 10,835 women, 449 women who had undergone menopause before 40 years of age were excluded (4.1% of overall sample) since premature ovarian failure has a different etiology than typical menopause and this exclusion also has the effect of preventing underestimation of a true ANM[10]. Women who reported undergoing menopause after age 62 were also excluded[12], as were women who did not experience menopause by age 62 (0.79% of overall sample) because some women might have pathologic conditions resulting in very late menopause or these values could be inaccurate[11]. Women diagnosed with breast, ovarian, or endometrial cancers (23% of overall sample) were excluded from the analysis since treatment for these female cancers includes hormone use, which might mask the true age of menopause[13, 14]. Women were also excluded if they had undergone surgical menopause by means of a hysterectomy (14.8% of overall sample) as their menopausal status is unclear; whilst it would have been legitimate to include women who had a hysterectomy in this analysis, no information on the age at which this surgery was performed had been collected.

Women who had missing information on ANM were also excluded. After these exclusions (28.8% of overall sample), 7,719 women remained for analysis as seen in Figure 1.

Assessment of Outcome

Menopausal status was assessed by asking the following question: "Have you gone through menopause, meaning that your menstrual periods stopped for at least one year and did not restart?" Women who answered "yes" were classified as *naturally post-menopausal*. Age at natural menopause is defined as the age of last menses, occurring after at least 12 months of amenorrhea as per the WHO's definition. Individual age at natural menopause, the primary outcome of this analysis, was reported in response to the following question: "How old were you when your menstrual periods stopped for at least one year and did not re-start?" Answers were recorded as age in years. Age at menopause was defined in this analysis as a continuous variable taking into consideration women who did not attain menopause at the time of outcome assessment. Self-reported age at menopause has shown in previous studies to have showed high validity and reliability, with over 70% of women recalling their age at menopause to within one year, after a time span of 7 years since asked, and an overall Spearman's correlation coefficient of 0.87[16].

Covariates

Sociodemographic variables included age at time of interview, ethnicity classified as white, aboriginal, and mixed/other, where "other" included non-white ethnic groups such as South Asian, Chinese, Hispanic, Arab, and Black. Additional sociodemographic information included highest education level (less than high school, high school to some college, Bachelor's degree or higher), marital status (partner vs. no partner), current employment status, and annual household income in CAD (<20,000, 20,000-50,000, 50,000-100,000, >100,000). Lifestyle factors included smoking status (never, former, or current), alcohol consumption (never, less than weekly, at least weekly), and frequency of leisure time physical activity in the past year (regular vs. non-regular) with regular physical activity defined as those who participated in leisure time physical activity at least once a day. Clinical variables included self-reported status of type 2 diabetes, hypertension, CVD, mood disorders, anxiety disorders, and presence of any allergies. Height and weight were also reported and were used to calculate BMI in kg/ m^2. A cut-

off of 20kg/m^2 was used for a BMI of underweight instead of 18.5 kg/m^2, because most of the women in this sample were older (45 and over), and so are less likely to be very underweight[12,20].

Endpoints

ANM was the main outcome, but the analysis method allowed premenopausal women to contribute person-years even if they did not reach natural menopause during the study period. Women who did not experience menopause were considered as censored observations (20.9% of overall sample). Endpoint age was defined as one of the following: a) age at natural menopause for postmenopausal women, b) age at interview if a woman was premenopausal, c) age at HRT initiation among women who had used HT prior to menopause.

Statistical Analysis

Nonparametric Kaplan–Meier cumulative survivorship estimates were used to assess the median ANM. The Kaplan–Meier method may be applied to cross-sectional data by using age rather than time as the censoring variable[19, 20]. Unadjusted and adjusted Cox proportional hazard regression models were then used to estimate the associations of ANM with the independent variables. Hazard ratios (HR's) and their confidence intervals (CI's) for earlier age at menopause were estimated. Exposures with HR's greater than 1 were associated with earlier onset of menopause, and those with HR's less than 1 were associated with later age at menopause. Validity of the proportional hazards assumption was checked by log-log plots with most variables not violating the assumption with the exception of: household income, smoking status, and CVD status. However, upon close inspection of the plots, the curves were close to parallel, with a minimal effect on the interpretation of the results (Appendix B). Moreover, based on prior research those variables were important factors, therefore the model was left unaltered. Goodness of fit of the multivariate Cox model was also assessed using the likelihood ratio test and Cox-Snell residuals to create the Nelson-Aalen cumulative hazard function (Appendix B). Our data overall followed an exponential distribution indicating that our model fits the data well[21]. Inverse probability weights (inflation and analytic weights) were used to account for the complex sampling method as per the guidelines set by CLSA. A p-value < 0.05 was considered statistically significant. All statistical data analyses were carried out using STATA version 12.

3.3 Results

Table 1 shows descriptive characteristics of the 7,719 women (weighted N= 5,065,656) included in this analysis. Their mean age at interview was 61.7 years (SD=10.6). Natural menopause was reported by 72.7% (weighted N= 3,675,924) of women. The majority of women in the sample were White (93%), and with partner (68.3%). Over 50% of women had a high school to some college education, were not currently employed, and over a quarter had a household income between 50,000-100,000 CAD. The proportion of women who had never smoked was 35.1%, while 54.5% were former smokers. Almost 50% of women consumed alcohol at least weekly, and over half performed leisure time physical activity on a regular basis in the past year. Around 70% of women in the sample had never used hormone therapy (HT). The proportion of women whose BMI was considered normal weight was 41.1%, whereas 30% of women were overweight and 22% were obese. Over a quarter of women reported to have hypertension, while less than 15% reported having type 2 diabetes or CVD, respectively. Figure 2 shows the distribution of their reported menopausal ages (Kaplan-Meier median age: 51 years; mean age: 49.9 years, range: 40–62 years). Table 2 outlines results of the unadjusted and adjusted Cox regression analysis. The multivariate model showed that women with no partner (HR= 1.09, 95%CI=1.02-1.17), with high school to some college level education (HR=1.13, 95%CI=1.06-1.21); women whose household income was between 20,000-50,000 CAD were at an increased risk of having an earlier natural menopause versus their counterparts. Current smokers had a greater hazard (HR=1.34, 95%CI=1.19-1.50) of having an earlier natural menopause than never smokers. Women who had CVD (HR=1.12, 95%CI=1.00-1.24) were also at a higher risk of having an earlier natural menopause than their counterparts. On the contrary, women who were currently employed (HR=0.84, 95%CI=0.79-0.90), who consumed alcohol at least weekly (HR=0.89, 95%CI=0.81-0.98) or less than weekly (HR=0.90, 95%CI=0.82-0.98) were more likely to have a later age at natural menopause. Moreover, women who were obese (HR=0.89, 95%CI=0.82-0.96**)** were more likely to have a later menopause than normal weight women in the multivariate model.

3.4 Discussion

This study aimed to identify the median ANM and investigate the characteristics affecting ANM among women in Canada. Median ANM was 51 years, as per the Nonparametric Kaplan–Meier cumulative survivorship estimates. Results of the multivariate analysis revealed associations between marital status, household income, education level, employment, smoking status, drinking habits, BMI, and CVD with age at onset of menopause. Elucidating the median age of menopause in a population is of clinical relevance given the potential adverse health outcomes associated with an earlier or later menopause. Identifying modifiable factors of ANM would thus impact etiological research on, and prevention of, these chronic diseases.

The results obtained by the Kaplan–Meier cumulative estimate indicated that overall median age at menopause was 51 years, which is similar to that found in previous studies[16, 20, 22]. The median age at menopause in Canada was similar to Australia[23], The Netherlands[24], and Greece[25], but was one year lower than that found by the most recent analysis from the US based Study of Women's Health across the Nation (SWAN) cohort[26]. Moreover, Schoenaker et al[6], in a meta-analysis of ANM that included 36 studies, found a mean ANM ranging from 46 to 52 years. ANM varied between geographical regions with African, Latin American, Asian and Middle Eastern countries having a lower ANM, while Europe, Australia, and USA having a higher ANM.

These large data provide evidence that socioeconomic conditions are key factors related to ANM, a finding consistent with previously published results[7, 8, 15, 27, 28]. Indeed, a meta-analysis including worldwide studies showed that lower education level and low occupation status were significantly associated with earlier ANM[6]. Employment, education, and income level are indicators of perceived economic security as well as social and emotional stress[29]. It has been suggested that psychological stress affects ovarian function and, therefore, age at menopause by lowering telomerase activity, a cellular enzyme that may act as a marker of ovarian age via its association with follicular atresia[29]. Physiological stress responses such as that of the hypothalamic-pituitary-adrenal axis, on the other hand, may also influence ANM[30].

Our results indicated that women without a partner were more at risk of having an earlier ANM compared to women with partner. Marital status has not been consistently measured in previous studies, with the exception of Gold et al[22], therefore not many explanations exist for this observed association. However, parity, a possible proxy indicator for marital status, has been consistently associated with older age at menopause[8]. Mishra et al[31] in a pooled study that consisted of 51,450 postmenopausal women from nine observational studies in the UK, Scandinavia, Australia and Japan, found that nulliparous women were at a significantly increased risk of early menopause (RR=1.32, 95%CI=1.09–1.59). This relationship provides support to the hypothesis that the rate of depletion of oocytes influences the age at menopause with increasing number of children delaying age at menopause[8, 22].

Prior studies have consistently shown that current smoking is associated with an earlier menopause[1, 6, 8-18, 22-26], as was observed in our study. Polycyclic aromatic hydrocarbons in cigarette smoke are non-reversibly toxic to ovarian follicles[32], thereby accelerating the onset of menopause by reducing circulating estrogen. Former smokers were also found to be at an increased risk of earlier menopause than never smokers. ANM in ex-smokers was rarely reported across studies[6, 33], with Schoenaker et al[6] reporting no difference in ANM between former and non-smokers. This study also indicated that alcohol consumption is associated with later onset of menopause. A positive association between alcohol consumption and ANM has previously been noted in the United Kingdom[11] and United States [8,10,14,22,26,34,35], but reports from other countries, including China, Norway, and Germany, have shown no association[20, 36-39]. Taneri et al[40] in a recent meta-analysis of 22 articles found that in prospective studies, a low-to-moderate alcohol intake (0–8 g/day (RR = 0.95; 95% CI: 0.93–0.98), and 16 g/day (RR = 0.89, 95%CI: 0.86–0.92) was associated with a later menopause. An explanation for this finding is that moderate alcohol consumption increases estrogen levels in premenopausal women[41].

The association of higher BMI with later ANM found in this study is in concordance with previous studies[13, 24, 26, 36]. However, the effect of BMI on age at menopause remains vague with other studies finding no association between BMI and ANM[14, 18, 22]. It is hypothesized that increased estrone production in the adipose tissue of obese women might delay menopause onset[42]. Estrone is formed by the peripheral aromatization of androgens, namely

androstenedione, which is secreted by the ovaries and/or adrenal glands[43]. The conversion of androgens into estrone has been linked to an increase in body weight[44]. On the other hand, since obesity has also been associated with inadequate ovarian function, it may be a determinant of earlier menopause[45]. An explanation to this observation might lie in that caloric restriction and nutritional deficiencies in both primates and humans are associated with amenorrhea[46].

Many chronic diseases have been linked to menopause onset[47-49]. Our results showed that women with a CVD diagnosis were at an increased risk of earlier menopause. This finding is interesting since comorbid health conditions such as CVD were not extensively assessed in relation to ANM in previous studies[16, 22]. The relationship and direction between CVD risk factors and outcomes vis a vis ANM is not clear. CVD could be a risk factor for ANM as shown in this study, which is consistent with Gold et al[22]. CVD risk factors such as total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c) levels, and systolic and diastolic blood pressure levels may be associated with menopausal status independent of age as de Kat and colleagues[50] found. Moreover, Kok et al[51], using data from the Framingham Heart Study cohort, showed that each 1% increase in the Framingham risk score pre-menopause was significantly associated with a 1.8 year decrease in ANM. Yet, CVD could also be an outcome of earlier ANM[52, 54]. Further research is needed to confirm the association between CVD and ANM, however. One mechanism through which menopause might affect CVD lies in estrogen deficiency. Estrogen is thought to protect against CVD and its risk factors in premenopausal women via both systemic and local vascular effects as it has been shown to lower LDL cholesterol and increase HDL cholesterol[53, 54]. After menopause, estrogen deficiency may lead to rapid increases in blood pressure[55] and vascular injury [56].

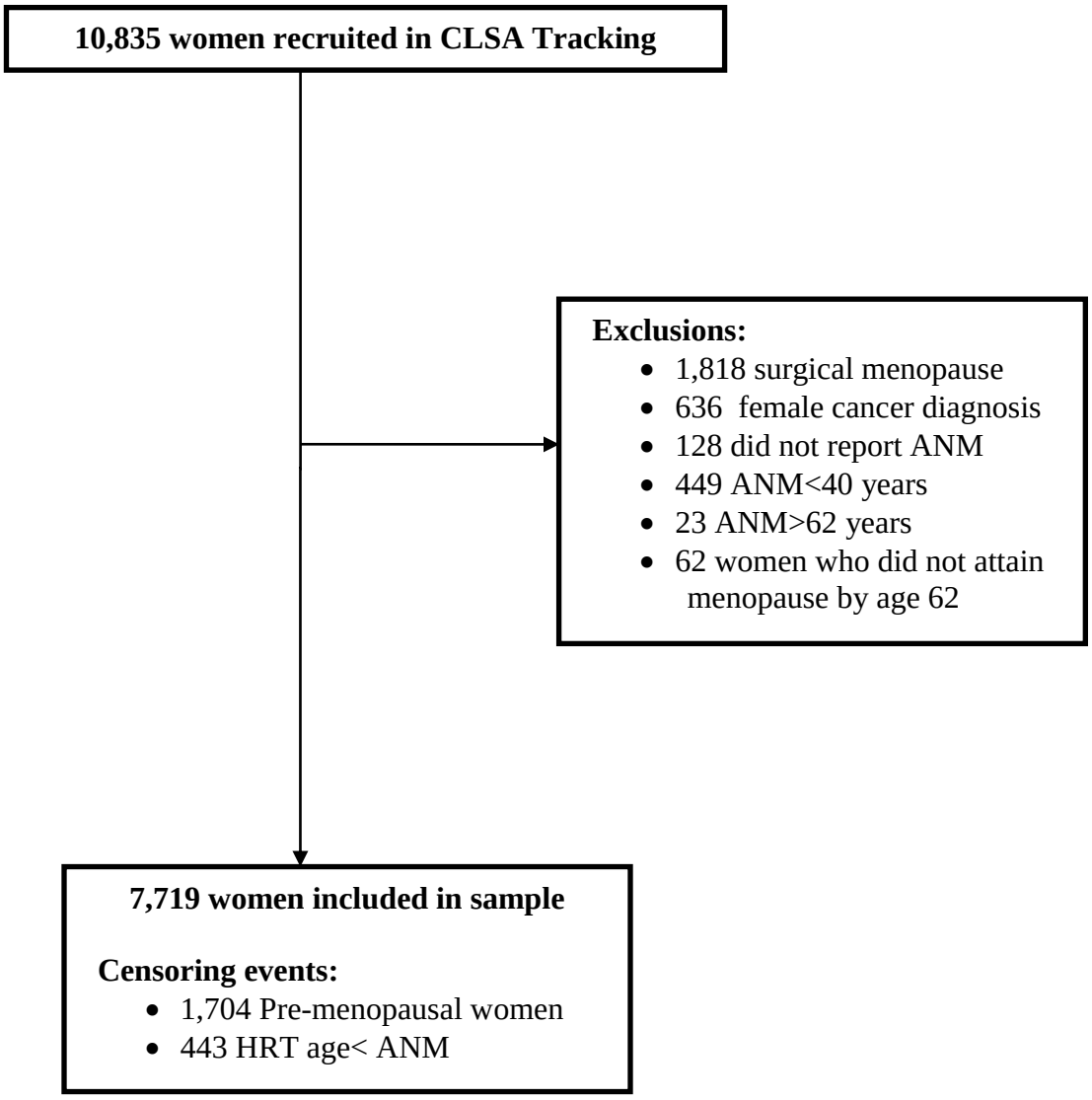
This study had several strengths. It is the first study to obtain a robust median estimate as well as the correlates of ANM in Canada using a large and nationally representative sample of participants. Furthermore, the simultaneous adjustment of multiple factors in the Cox model, while censoring at initiation of hormone therapy, limited the potential for confounding bias due to HRT use. Nonetheless, a few limitations exist. One, the cross-sectional nature of the study precludes the inference of causality. Recall bias due to self-report of ANM might be another limitation. Although all cases with chronic diseases were diagnosed by a physician, these

diagnoses were not confirmed by more objective medical procedures. In addition, most of the data were derived from questionnaires based on memory recall. Data on oral contraceptive use, breastfeeding, age at menarche, and early life circumstances- factors that have been previously linked to age at menopause- were not collected. Lastly, the exclusion of women with surgical menopause and breast cancer might affect the generalizability of our findings.

3.5 Conclusion

This study constitutes the first nation-wide study of the onset of natural menopause in Canadian women. The overall median ANM was 51 years. This study's findings revealed that the ANM reflects the complex intertwining of social determinants, lifestyle factors and health conditions. Since ANM is a risk factor for a number of disorders, understanding factors associated with menopausal age is integral to identify menopausal women who are at high risk for future morbidity. CVD status was significantly associated with ANM highlighting the importance of both control and prevention of CVD risk factors during the menopausal transition. Prospective studies are required to determine the causal association between chronic conditions, such as CVD, as well as lifestyle factors and ANM. The evidence provided here also emphasizes early preventive strategies and clinical surveillance of chronic diseases for women in the menopause transition.

Figure 3.1: CLSA participants' flowchart



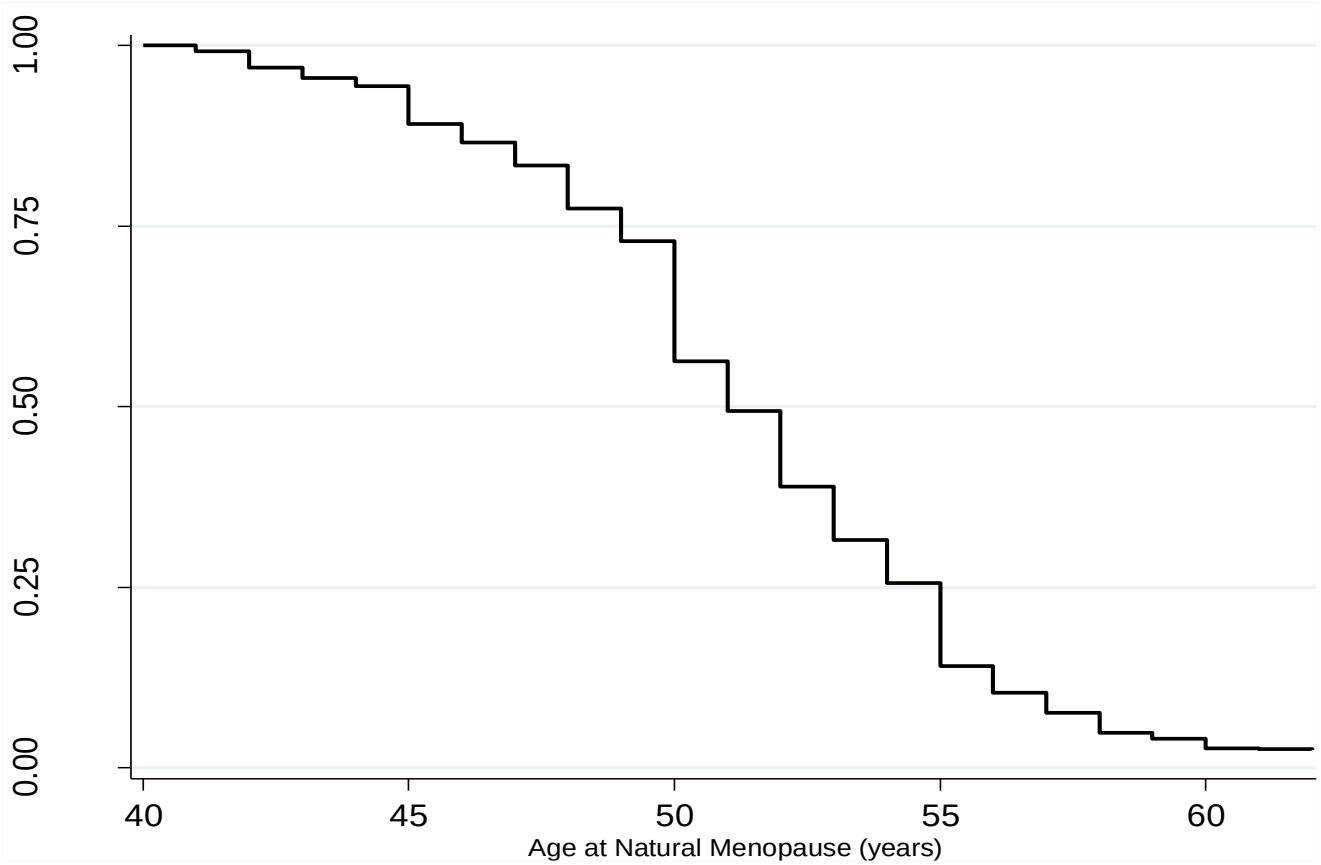


Figure 3.2: Kaplan–Meier cumulative estimates of ANM in Canadian women.

Table 3.1: Baseline characteristics of women in the CLSA by menopause status.

	Total Sample		**Natural Menopause**		**Median age at natural menopause, years (IQR)[c]**
Variables	**N[a]**	**(%)**	**N**	**(%)[b]**	
Sociodemographic					
Ethnicity					
White(non-aboriginal)	4,613,943	93.0	5,163	73.3	51(49-54)
Aboriginal	145,446	2.90	150	63.6	51(49-55)
Mixed/other	201,149	4.05	160	65.7	51(49-54)
Marital Status					
No partner	1,583,493	31.5	1,970	82.2	50(48-54)
With partner	3,449,925	68.5	3,599	68.3	52(50-55)
Education Level					
Less than high school	381,148	7.60	428	75.8	51(49-55)
High school to some college	2,776,595	55.2	3,270	75.9	51(48-54)
Bachelor's degree or higher	1,875,833	37.3	1,867	67.0	52(50-55)
Employment Status					
Not currently working	2,648,432	52.6	3,630	90.0	51(48-54)
Currently working	2,385,104	47.4	1,939	53.5	52(50-55)
Household Income (CAD)					
<20,000	273,551	5.80	378	88.6	50(47-53)
20,000-50,000	1,176,701	25.1	1,615	86.7	51(48-54)
50,000-100,000	1,619,610	34.6	1,841	74.5	52(49-55)
>100,000	1,614,550	34.5	1,240	53.6	52(50-55)
Lifestyle					
Smoking Status					
Never	1,760,239	35.1	1,896	70.3	52(50-55)
Former	2,750,097	54.9	3,084	74.2	52(49-55)
Current	500,040	10.0	562	72.2	50(47-53)
Alcohol consumption					
Never	655,846	13.0	888	80.4	50(48-54)
Drinks less than weekly	1,829,020	36.4	2,118	71.6	51(49-55)
Drinks at least weekly	2,544,592	50.6	2,557	71.0	52(49-55)
Leisure time physical activity					
Non-regular	2,237,806	44.5	2,629	74.9	51(49-54)
Regular	2,789,076	55.5	2,927	70.6	52(49-55)
HT use					
Never	3,558,393	70.9	3,561	65.1	52(50-55)
Ever	1,463,020	29.1	1,995	91.5	50(48-54)

Table 3.1 (Cont'd): Baseline characteristics of women in the CLSA by menopause status.

Health related					
BMI (Kg/m^2)					
20.0-24.99 (normal weight)	2,080,942	41.7	2,142	70.1	51(49-54)
<20.00 (underweight)	322,450	6.50	336	71.9	50(49-54)
25.0-29.99 (overweight)	1,502,085	30.1	1,782	75.8	51(49-55)
> 30.0 (obese)	1,081,789	21.7	1,268	73.3	52(49-55)
Hypertension					
No	3,565,264	70.9	3,626	67.1	52(49-55)
Yes	1,464,920	29.1	1,938	85.6	51(48-54)
Diabetes					
No	4,432,514	88.1	4,812	71.4	51(49-55)
Yes	599,739	11.9	753	81.4	51(48-54)
Cardiovascular disease					
No	4,610,836	91.7	5,026	71.2	51(49-55)
Yes	415,695	8.30	530	88.5	50(47-53)
Allergies					
No	2,853,193	56.8	3,193	73.4	51(49-54)
Yes	2,171,697	43.2	2,362	71.6	52(49-55)
Mood Disorders					
No	4,146,876	82.4	4,606	72.7	51(49-54)
Yes	885,077	17.6	961	72.1	51(49-54)
Anxiety Disorders					
No	4,562,525	90.7	5,084	73.0	51(49-54)
Yes	466,394	9.30	483	69.1	51(49-55)

Abbreviations: IQR, interquartile range.

a Sample size is estimated using inflation weights; figures not always sum up to the total due to missing values.

[b] Frequencies are column percentages estimated using analytic weights.

[C] Calculated using Kaplan-Meier estimate

Table 3.2: Unadjusted and adjusted hazard ratios (HR's) for age at natural menopause (ANM).

	Unadjusted Hazard Ratio	**Adjusted Hazard Ratio**
Variables		
Sociodemographic	HR(95%CI)	HR(95%CI)
Ethnicity		
Aboriginal	1.13(0.95-1.35)	1.03(0.85-1.23)
Mixed/other	1.11(0.93-1.32)	1.17(0.96-1.41)
White	1.00	1.00
Marital Status		
No partner	**1.21(1.14-1.28)**	**1.09(1.02-1.17)**
Education Level		
Less than high school	1.07(0.96-1.20)	1.02(0.91-1.15)
High school to some college	**1.20(1.13-1.27)**	**1.13(1.06-1.21)**
Bachelor's degree or higher	1.00	1.00
Employment Status		
Currently working	**0.80(0.75-0.85)**	**0.84(0.78-0.90)**
Household Income (CAD)		
<20,000	**1.50(1.32-1.70)**	1.10(0.95-1.28)
20,000-50,000	**1.36(1.25-1.47)**	**1.14(1.04-1.25)**
50,000-100,000	**1.18(1.09-1.28)**	**1.10(1.01-1.19)**
>100,000	1.00	1.00
Lifestyle		
Smoking Status		
Never	1.00	1.00
Former	1.05(0.99-1.12)	**1.07(0.99-1.14)**
Current	**1.42(1.27-1.57)**	**1.34(1.19-1.50)**
Alcohol consumption		
Never	1.00	1.00
Drinks less than weekly	**0.88(0.81-0.95)**	**0.90(0.82-0.98)**
Drinks at least weekly	**0.85(0.78-0.92)**	**0.89(0.81-0.98)**
Leisure time physical activity		
Regular	**1.09(1.03-1.15)**	1.03(0.97-1.10)
Health related		
BMI (Kg/m^2)		
20.0-24.99 (normal weight)	1.00	1.00
<20.00 (underweight)	**1.15(1.02-1.30)**	1.13(0.99-1.30)
25.0-29.99 (overweight)	0.99(0.92-1.06)	0.94(0.87-1.01)
> 30.0 (obese)	0.96(0.90-1.04)	**0.89(0.82-0.96)**
Hypertension		
Yes	**1.12(1.06-1.19)**	1.06(0.99-1.13)
Diabetes		
Yes	1.08(0.99-1.18)	0.98(0.89-1.07)
Cardiovascular disease		
Yes	**1.23(1.12-1.36)**	**1.12(1.00-1.24)**
Allergies		
Yes	0.96(0.91-1.02)	0.99(0.94-1.05)
Mood Disorders		
Yes	1.02(0.94-1.96)	1.02(0.94-1.11)
Anxiety Disorders		
Yes	0.98(0.88-1.08)	0.92(0.82-1.04)

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Chapter 4

Hormone Therapy Use in the Canadian Longitudinal Study on Aging (CLSA): A Cross-Sectional Analysis

Abstract

Objective: To assess the prevalence and factors associated with hormone therapy (HT) use among Canadian women.

Methods: Baseline data from the Tracking cohort of the Canadian Longitudinal Study on Aging (CLSA) was used for this analysis. The main outcome was HT use among women aged 45-85 years, defined as current, past, and never users. Multinomial logistic regression models were used to examine the differences between current, past and never HT users in terms of sociodemographic, health behavior and health-related variables.

Results: Overall, 9.5% of the sample reported current use of HT, while 21.9% reported past use. The main factors associated with a lower likelihood of current HT use were: older age (>80 y), non-white ethnic background, current employment, regular smoking, obesity, and breast cancer. By contrast, alcohol consumption, and the presence of allergies or mood disorders were positively associated with current HT use.

Conclusions: These findings provide a recent national picture of HT use in Canada that may be used to inform opportunities for improved physician–patient communication regarding menopause management.

Keywords: Menopause, Hormone Therapy, Prevalence, Canada

4.1 Introduction

Vasomotor symptoms (VMS), encompassing night sweats and hot flashes, are experienced by 80% of women during the menopausal transition[1]. Recent data suggest that VMS persist for a mean duration of 7.4 years[2], with over 30% of women experiencing VMS for 10 years or more[2-5]. Hormone therapy (HT) is currently the most effective strategy for managing VMS. HT contains estrogen, and in women with an intact uterus (i.e. not having undergone hysterectomy), it is combined with a progestogen for endometrial protection from hyperplasia[6].

HT use has fluctuated over the past 60 years owing to changes in its risks and benefits. In the late 1990s, the prevalence of hormone use exceeded 40% among women aged 50–69 years in the US[7]. In 2002, the Women's Health Initiative (WHI) trial revealed an increase in thrombotic risk and a slight increase in breast cancer risk among older users of oral estrogens and progestogens that subsequently contributed to a worldwide reduction in HT use[8]. Total HT use has been declining ever since 2002; from 29.8% to 15.3% in 2004, with these trends persisting up to 7 years post WHI[9]. Due to the WHI study results, prescription behaviors and indications for HT changed, except for its beneficial impact on osteoporosis[9]. In fact, the clinical guidelines at the time of this publication recommend HT use for relief of menopausal symptoms while individualizing therapy based on clinical factors such as venous thrombosis, breast cancer, stroke and coronary heart disease (CHD) risk, as well as the patient's quality of life priorities[10].

Studies that investigated the characteristics of women's HT use after 2002 remain scarce[11-15]. Younger age, higher educational level, postmenopausal status, African American and Hispanic race, and being married have been associated with HT use, however not all of these associations are consistent or conclusive[11-16]. Little research has been done on HT use in Canada[17, 18], and of those that have, few studies have reported a reduced frequency of HT use after 2002. For example, De et al[18], using data from the National Population Health Survey (NPHS), showed that total hormone use among women aged 50–69 years was 29.8% at the beginning of 2002 but decreased to 15.3% in 2004 after the publication of the WHI findings. VMS impair women's quality of life[19], and unmanaged VMS have been linked to physical health outcomes including cardiovascular disease (CVD) with studies revealing an association between

VMS and CVD risk factors and subclinical CVD[20, 21]. Most studies in Canada did not examine the characteristics of women who use HT. Furthermore, no updated national estimates of the use of HT and its types have been published since the WHI era. A more recent description of the prevalence of HT use is important to properly understand whether women are abiding by the guidelines for HT use. Given the changing landscape of menopause management, this information will provide important insight into the use and monitoring trends of HT use. Furthermore, identifying the extent to which individual sociodemographic, medical, and behavioral characteristics are associated with the use of HT might help reveal ways of improving women's decision making regarding HT. Given the paucity of information that characterizes current HT use, this study aims to assess the prevalence and factors associated with HT use among Canadian women.

4.2 Methods

Study Design and Sample

Baseline data from the Tracking cohort (version 3.0) of the Canadian Longitudinal Study on Aging (CLSA) was accessed for secondary analysis. In short, the CLSA is a 20 year prospective study of 50,000 men and women aged between 45 and 85 years at baseline. Launched in 2010, the CLSA aims to provide national-level insight into the behaviors and health of adults ages 45 to 85 years at baseline. The study has two categories of participants: i) the Tracking cohort who were randomly selected within age and sex strata in each province and followed by telephone interview, and; ii) the Comprehensive cohort participants, who had more intensive physical measures as well as questionnaire surveys. The Tracking cohort included n=21,241 (M: 10406; F: 10835) free-living participants distributed across all 10 provinces. Individuals living in long-term care institutions and having cognitive impairment at the time of recruitment were excluded at baseline. However, those living in households and transitional housing arrangements (e.g., seniors' residences, in which only minimal care is provided) were included. The CLSA study design has been previously described in detail elsewhere[22, 23].

Assessment of Outcome

The main outcome variable was current HT use. Women using HT when interviewed in 2010-2013 were defined as current users, women who had used HT in the past were labelled as past users, while those who had never taken HT were labeled as never users. Current HT use was created using the following variables: age at the time of interview, duration of HT use and age at initiation of HT use. Women who did not provide complete information to define current use of hormone therapy, and whose age at HT initiation was < 40 years were excluded from this analysis as it is possible that they confused hormone therapy indicated for menopause with oral contraception, or they may have been diagnosed with primary ovarian insufficiency (premature menopause)-a rare condition that indicates hormone use at an earlier age than the majority of women who experience normal menopause[24]. Therefore, out of the 10,835 women enrolled in this study, 694 (6.4% of sample) were excluded from the analysis. A previous study based in Denmark on women aged 50-59, found a validity of over 70% for self-reported HT use when compared with population-based prescription databases[58]. Lastly, no data were collected regarding the presence of VMS, and information regarding the main reasons for using HT.

Covariates

Sociodemographic variables included age at time of interview (<50, 50-59, 60-69, 70-79, ≥80 years), ethnicity classified as white, aboriginal, and mixed/other, where "other" included non-white ethnic groups such as South Asian, Chinese, Hispanic, Arab, and Black. Additional sociodemographic information included highest education level (less than high school, high school to some college, Bachelor's degree or higher), marital status (partner vs. no partner), current employment status, and household income in CAD (< 20,000, 20,000-50,000, 50,000-100,000, 100,000-150,000, >150,000). Lifestyle factors included smoking status (never, former, or current), alcohol consumption (never, less than weekly, at least weekly), and frequency of leisure time physical activity in the past year (regular vs. non-regular). Those who answered "at least once per day," or "at least once per week," were considered regular participants, and those who answered "at least once per month," "at least once per year," or "never" were considered non–regular participants of physical activity. Information on health-related variables included menopausal status (pre-, natural, or surgical menopause (hysterectomy)), self-reported Body Mass Index (BMI: kg/m^2), self-reported status of type 2 diabetes, hypertension, cardiovascular disorders, mood disorders, anxiety disorders, allergies, and osteoporosis was included. A cut-off of $20kg/m^2$ was used for a BMI of underweight because most of the women in this sample were older (45 and over) and so are less likely to be very underweight. Female cancers were also measured and included breast, ovarian, and other gynecological cancers such as cervical and endometrial cancers.

Statistical Analysis

The prevalence of HT use was estimated through population weights and examined at the national level. Applying the appropriate sample weights to the CLSA data allowed the survey data to be representative of the sampled population. Additional analytic details are available at: https://www.clsa-elcv.ca/doc/1041. At the bivariate level, differences in the proportion of type of HT use, age at initiation, and duration of HT use were assessed among the different levels of HT use by chi-square tests. In order to assess the associations of each independent variable with HT use, bivariate and multivariable logistic regression models were used, with HT use in its binary form (current versus non-current users) as the dependent variable. Odds ratios (OR) and confidence intervals (95% CI) were presented for each variable in the model. Given that our aim

was to investigate factors associated with “current” HT use and no difference in sociodemographic characteristics was observed when “past” and “never” HT users were compared, a single pooled category for “non-current” users was created. To examine the differences between HT current, past and never users in terms of sociodemographic, lifestyle and health related variables, a multinomial logistic regression (MLR) was performed. The latter analysis has been preferred to the classical binomial regression analysis, since it enables estimates for current use without excluding past users from the analysis, nor does it join never and past users[13]. As a result of the multinomial regression model, two odds ratios were obtained for each variable, the first for the likelihood of being a current versus a never HT user and the second for the odds of being a past versus a never user. However, results regarding past HT users were not shown in table 2, since they were less relevant for the aim of the present analysis and, moreover, data for this group of women were not sufficiently reliable due to difficulty in elucidating the temporal sequence between HT use start and cessation and many of the variables investigated and due to the potential for reverse causality bias[13]. Inverse probability weights (inflation and analytic weights) were used to account for the complex sampling method as per the guidelines set by CLSA. STATA version 12.0 was employed for all analyses. Statistical significance for all analyses was set at alpha 0.05 for a two-tailed test.

4.3 Results

In total, 854 out of 10,141 (weighted n=6,426,271) women were included with 9.5% reporting current use of HT, 21.9% had used it in the past, and 68.6% were never users (results not shown). Age 50-54 years at initiation is the most common group for both current and past HT users (Table 1). Among current HT users, 45.6% were taking estrogen alone, 35.5% were on estrogen plus progesterone, 8% were using progesterone alone and 9.7% reported estrogen use via cream or gel. Table 2 highlights the unadjusted odds ratios of current HT use using binomial and multinomial analyses. Both analyses showed that as women aged they were significantly less likely to be current HT users. Women who had more than one ethnic background or were neither white nor aboriginal were less likely to be current HT users compared to women of a white ethnic background in both MLR and BLR (OR=0.43, 95%CI=0.21-0.89) models. In both analyses, former smokers, women who drink either at least weekly or less than weekly, who practiced physical activity regularly, who reported being naturally menopausal or having had surgical menopause, had allergies, or reported having a mood disorder, had a significantly higher odds of being a current HT user than their counterparts. On the other hand, women who were obese were less likely to report current HT use in both analyses (OR=0.65, 95%CI=0.52-0.80); results were similar for women with diabetes (OR=0.71, 95%CI=0.55-0.91). Women who had breast cancer were 54 % less likely to be current users compared to never users (OR=0.46, 95%CI=0.28-0.76), according to the MLR analysis. Results of the BLR analysis were identical to that of the MLR.

Table 3 shows the results of the multivariable analyses. The results of the MLR model indicate that the frequency of current HT use significantly decreased with increasing age (e.g. women >80 years, (OR=0.31 95%CI=0.15-0.63). Women who were of a non- white ethnic background (OR=0.32, 95%CI=0.14-0.73), currently employed (OR=0.76, 95%CI=0.61-0.94), current smokers (OR=0.69 , 95%CI=0.49-0.97), obese (OR=0.57 , 95%CI=0.44-0.75), and who had breast cancer (OR=0.43 , 95%CI=0.26-0.71) , were significantly less likely to be current HT users than their counterparts. On the contrary, women who regularly performed physical activity (OR=1.23,95%CI=1.03-1.46), who drank at least weekly (OR=1.94, 95%CI=1.44-2.61) or less than weekly (OR=1.51, 95%CI=1.13-2.02), who were post-menopausal (natural or surgical), who had any allergies (OR=1.29, 95%CI=1.08-1.54) or any mood disorders (OR=1.86,

95%CI=1.50-2.32) had a higher likelihood of being current HT users. No significant differences were observed according to marital status, education level, diabetes, CVD, ovarian cancer, or other female cancers, in both multivariable analyses.

4.4 Discussion

This study is the first nationally representative study to report on the prevalence and correlates of HT use in Canada. Around 10% of 45-85 year olds in the sample reported current use of HT. A non-white ethnic background, current employment, current smoker, obesity, and breast cancer were negatively associated with current HT use, whereas alcohol consumption, natural or surgical menopause, and the presence of allergies or mood disorders, were positively associated with current HT use. These findings provide a new benchmark of HT use in Canada and may inform clinicians about women who are not using HT that perhaps should be using it.

Our estimate of current HT use (9.5%) is similar to the prevalence obtained by a recent national online survey assessing HT use that was developed by the North American Menopause Society (NAMS) Advisory Panel. This survey, administered to 3,725 women who were postmenopausal or experiencing the menopause transition, found that 9% of women were current HT users[25]. The US based National Health and Nutritional Examination Survey (NHANES) reported that 11.9% of women aged 40 and older were currently using oral postmenopausal hormones in 2002-2003[26]. This estimate was obtained following publication of the WHI results in 2002 that indicated that the health risks of estrogen plus a progestogen outweighed its benefits among women with an intact uterus[27]. In 2004, results for the WHI trial of estrogen only among women with hysterectomy showed that this intervention was also associated with an elevated risk of stroke and pulmonary embolism. It was therefore concluded that each form of postmenopausal hormone was unsuitable for chronic disease prevention, with the exception of osteoporosis, in healthy women[27, 28]. As a consequence, the prevalence of current HT use in the NHANES continued to decline, and was at 4.7% for any type of HT in 2009–2010[26]. Our estimate was also comparable with that of other countries such as Australia, where Velentzkis et al[29] showed that 13% of older women were current HT users. Studies from other countries including Italy[13], Denmark[30], as well as Germany, the UK, France and Spain[31] reported HT use estimates higher (around 16%) than the current sample. It should be noted that differences in user patterns and knowledge of HT across continents exist - in addition to differences in cultural expectations about menopause, symptom perception, as well as health-care systems – that could collectively account for discrepancies in the estimates obtained. In Canada, the use of HT has

also undergone some notable changes over the past two decades. The 2006/2007 National Population Health Survey (NPHS) highlighted that 13.7% of postmenopausal Canadian women were currently taking or had recently taken any type of HT. This decline in HT use rates occurred very rapidly, decreasing from a prevalence of 29.8% in 2002 to a prevalence of 15.3% in 2004[18]. Similar findings were also reported among older (above 65) women in Ontario in late 2002[17]. Although differences in age ranges and survey methods must be taken into account, when taken together, these findings suggest that the overall prevalence of HT use in Canadian women has somewhat stabilized over the past decade, following an initial rapid fall after 2002.

Use of HT decreased with increasing age, as expected, since many women may have begun this therapy to treat VMS during the menopausal transition and discontinued it as symptoms resolved post-menopause. Ethnicity was associated with current HT use, whereby women of a non-white ethnic background were less likely to use HT. This finding is consistent with that of Gerber et al[32], and Brett and Reuben[12]. No relationship between hormone use, marital status, and education level was detected in our study. This is in contrast to other studies that describe these factors as correlates of hormone use[13, 32-34]. These variations in the results obtained are likely to be attributed to differences in methods of HT status definition and ascertainment, the age range considered, and the sampling frame employed by each study. Employment status was also associated with hormone use, where women who were currently working had lower odds of currently using HT. Few recent studies[13] have examined the link between employment and HT use; however, unlike our findings, several studies conducted in the 1990s found that a current job is an important positive determinant of current HT use[35-37]. The current relationship between occupation and HT use remains unclear and warrants further investigation since VMS that occur during the menopausal transition may pose some difficulties for women at work, and so HT can help women have a better quality of life at work by alleviating these bothersome symptoms.

Previous studies have supported the hypothesis of an overall healthier profile for HT users [13, 36] which is in concordance with our finding that current smokers were less likely to be HT users while women who exercised regularly were more likely to be current HT users. A positive predicting factor of HT use was alcohol consumption. Indeed, VMS are more frequent

among alcohol drinkers than non-drinkers[38]. In fact, recent data from the longitudinal Health and Social Support (HeSSup) Study in Finland[39] found that moderate or heavy drinkers were over 30% more likely to initiate HT than light drinkers or abstainers. Body weight has been reported as being related to HT use, whereby overweight and obese women being less likely to use HT[13, 33, 37], and the results of the present study corroborate this observation. The impact of body weight on the occurrence of VMS during menopause is not clear. Body fat is a supplemental source of estrogen because androstenedione (an androgen and precursor of estrogen) is aromatized into estrone in adipose tissue[41, 42]. As a result, body fat may also be considered a protective factor for VMS[23]. Thus, it is thought that women with higher adiposity have higher estrogen concentrations than thinner women and therefore, lower odds of developing VMS[44].

Several health conditions were associated with HT use. Interestingly, women who reported suffering any allergies were more likely to be current HT users than women with no allergies. A paucity of studies have outlined the association between atopy, asthma and HT use. Yet, there is evidence that respiratory symptoms and asthma are associated with HT use especially among women with a normal or low BMI[45]. In fact, studies suggest that use of HT improves the symptoms of asthma[46]. The longitudinal Respiratory Health in Northern Europe study, an international, multicenter study, found that the risk of new-onset asthma increased by more than two times in women in the menopause transition and who were early postmenopausal[46]. Thus, women with reduced respiratory function may be more likely to use HT given their declining estrogen levels due to menopause. Sex steroids affect immune regulation, with estradiol showing both pro-inflammatory and anti-inflammatory properties [47], which in turn might affect the presentation of asthma, wheezing, and allergic sensitization. Further research is needed to explain the role of HT use among women with atopy and asthma, as well as the intersection of the role of obesity in this relationship. In addition, women with mood disorders, such as depression and bipolar disorder, were more likely to use HT. Our results were similar to that indicated by Gerber et al[32], where women using the US veteran affairs care system with any mental health diagnosis were almost two times more likely to use HT. This relationship might be explained by the fact that high anxiety levels, depressive symptoms, and perceived stress[48], as well as negative mood (affect) [49] have been associated with an increased likelihood of VMS. The relationship between negative affect and VMS is bidirectional, as VMS also influence mood[50].

Low estrogen may be a risk factor for developing and maintaining Post Traumatic Stress Disorder (PTSD) after traumatic events[51]. Moreover, VMS can precede, follow, or occur concurrently with depression; however, not all women who experience VMS will also experience depression[52]. Thurston et al[53] indicated that women who suffered from different forms of childhood abuse or neglect were almost two times at a higher odds of having VMS during the menopausal transition as well as lower levels of serotonin. Therefore, estrogen therapy could positively impact serotonin receptor distribution rendering it a mood regulator[54, 55] which could encourage adherence to hormone therapy by women with mood disroders[55]. The relationships between negative affect and VMS are not fully understood, and may involve a complex interplay between physiologic factors, such as hormones, neurotransmitters, and sleep disorders[54], and psychological factors, such as personality type[49].

Finally, women reporting breast cancer were significantly less likely to currently use HT (OR=0.43, 95%CI=0.26-0.71) which is in accordance with previous studies[32, 37]. It has been established that long-term HT use leads to a small increase breast cancer risk [56]. Recent data suggest that women who initiate HT soon after menopause have a higher breast cancer risk than those who start treatment later[57]. For this reason, HT use among women with breast cancer is not recommended[13]. Therefore, the prevalence of hormone consumption among women with breast cancer would have been expected to be lower than women with no breast cancer. Another reason for the low rate of HT use among breast cancer cases may be due to hormonal therapy and/or aromatase inhibitors being the protocol treatment[42, 56], and the use of drugs such as tamoxifen, an estrogen receptor modulator that acts as an estrogen antagonist by competitively binding to estrogen receptors on tumors.

Strengths of this study include that it is the first to present a detailed description of the patterns of HT use to date in Canada. Second, use of a large and nationally representative sample increases the generalizability of our findings. Furthermore, the number and variety of different sociodemographic, health behavior, and other variables controlled for in analysis limits the potential for confounding bias. Nonetheless, a few limitations exist. First, the potential for reverse causality in some variables is probable; for example, some women might chose to start exercising after initiating HT. Second, the cross-sectional nature of the study precludes the

inference of causality. Due to the self-report nature of HT use, recall and reporting bias may also be present. The percentage of ethnic diversity in the CLSA is relatively low, therefore our results can mostly be generalized to whites. Lastly, some associations such as the relationship between allergy and HT use may have arisen due to chance, given that many variables were examined in the multivariable model.

4.5 Conclusion

Results of the present study on a large sample of Canadian adult females estimated that current HT use is 9.5%. On the whole, the likelihood of current HT use increased with an overall healthier profile than never HT use. The main factors associated with current HT use were older age, a non-white ethnic background, current employment, smoking, alcohol consumption, obesity, and the presence of allergies, mood disorders, and breast cancer. Prospective, longitudinal studies will better inform our understanding of HT use over time. Mixed methods designs examining women's experience of VMS, as well as decision- making around HT use and discontinuation, could help explain the rates found in this study. Ascertainment of providers' knowledge and prescribing patterns will also yield key information to understand HT use, as it is important that HT be given after a thorough and systematic risk/benefit assessment. Finally, effective doctor patient communication and programs to increase women's awareness and education about menopause management are essential to improve women's understanding, compliance, and ability to cope with the symptoms accompanying the menopausal transition.

Table 4.1: Distribution of hormone therapy (HT) use characteristics in the sample.

	HT use						
Age at HT initiation	**Total Sample N**	**%[a]**	**Past* N**	**%[b]**	**Current* N**	**%[b]**	P-value
<45 years	300,560	4.40	374	16.8	106	11.9	<0.001
45-49 years	632,740	9.30	712	32.0	264	29.8	
50-54 years	747,769	11.0	800	35.9	332	37.4	
>54 years	360,146	5.30	343	15.4	185	20.9	
HT use duration							
<1 year	580,172	24.7	666	28.1	204	21.5	<0.001
1-4 years	508,942	21.7	534	22.4	226	24.0	
5-9 years	460,231	19.6	555	23.3	174	18.4	
>9 years	796,565	34.0	624	26.2	342	36.2	
HT type							
Estrogen and progesterone	725,126	35.5	762	37.9	301	33.4	<0.001
Estrogen	932,549	45.6	967	48.1	346	38.4	
Progesterone	163,695	8.00	131	6.50	84	9.30	
Estrogen cream or gel applied to skin	197,350	9.70	143	7.10	142	15.8	
IUD with progesterone	24,882	1.20	8.0	0.40	28	3.10	

[a] Sample includes never HT users, and is estimated using inflation weights; figures not always sum up to the total due to missing values.

[b] Frequencies are column percentages estimated using analytic weights.

* Not including never HT users

Table 4.2: Prevalence of HT never, past and current users by various variables and results of the unadjusted multinomial analysis for current HT use.

	Never[a]	**Past[a]**	**Current[a]**	**Current[b]**
	%	%	%	Unadjusted OR (95%CI)
Variables	**N (%)**	**N (%)**	**N (%)**	
Sociodemographic				
Age (years)				
<50	1,252(17.8)	17(0.8)	80(9.0)	1.00
50-59	3,097(44.1)	332(14.9)	430(48.5)	**2.18(1.61-2.94)**
60-69	1,502(21.4)	1,033(46.4)	265(29.9)	**2.77(2.04-3.77)**
70-79	798(11.4)	684(30.7)	95(10.8)	**1.88(1.32-2.66)**
≥80	377(5.4)	163(7.3)	16(1.8)	0.67(0.39-1.15)
Ethnicity				
White(non-aboriginal)	6,356(92.5)	2,127(96.7)	830(94.4)	1.00
Aboriginal	238(3.5)	46(2.1)	37(4.2)	1.31(0.87-1.98)
Mixed/other	274(4.0)	26(1.2)	13(1.4)	**0.43(0.21-0.89)**
Marital Status				
With partner	4771(67.9)	1413(63.4)	603(68.0)	1.00
No partner	2255(32.1)	816(36.6)	283(32.0)	1.01(0.85- 1.19)
Education Level				
Less than high school	514(7.3)	181(8.1)	63 (7.1)	1.00
High school to some college	4,069(57.9)	1354(60.8)	508 (57.5)	1.02(0.77-1.36)
Bachelor's degree or higher	2,440(34.7)	692(31.1)	314 (35.5)	1.05(0.78-1.42)
Employment Status				
Not currently working	3,441(49.0)	1,852(83.1)	502 (56.6)	1.00
Currently working	3,584(51.0)	376(16.9)	385 (43.4)	**0.74(0.63-0.86)**
Household Income (CAD)				
<20,000	447(6.9)	150(7.5)	58 (7.1)	1.00
20,000-50,000	1739(26.9)	743(37.0)	179 (22.0)	0.75(0.54-1.03)
50,000-100,000	2126(32.9)	764(38.0)	296 (36.3)	1.05(0.77-1.45)
100,000-150,000	1239(19.2)	227(11.3)	156 (19.2)	1.10(0.78-1.55)
>150,000	916(14.2)	125(6.2)	127(15.5)	1.25(0.87-1.81)
Lifestyle				
Smoking Status				
Never	2,582(36.9)	669(30.2)	282(31.9)	1.00
Former	3,602(51.5)	1348(60.8)	523(59.1)	**1.22 (1.03-1.44)**
Current	807(11.5)	199(9.0)	79(9.0)	0.90(0.68-1.21)
Alcohol consumption				
Never	1,171(16.7)	352(15.8)	98(11.1)	1.00
Drinks less than weekly	2,843(40.5)	792(35.5)	316(35.6)	**1.34(1.05-1.72)**
Drinks at least weekly	3,004(42.8)	1085(48.7)	471(53.2)	**1.78(1.40-2.27)**
Leisure time physical activity				
Non-regular	3,416(48.7)	1,035 (46.6)	373(42.0)	1.00
Regular	3,602(51.3)	1,185(53.4)	512(58.0)	**1.27(1.09-1.49)**

Table 4.2(Cont'd): Prevalence of HT never, past and current users by various variables and results of the unadjusted multinomial analysis for current HT use.

Health related				
Menopause Status				
No	2,033(29.1)	64 (2.9)	101(11.5)	1.00
Natural	4,243(60.6)	1680(75.4)	574(65.4)	**2.02(1.56-2.61)**
Surgical (Hysterectomy)	722(10.3)	486(21.8)	203(23.2)	**3.51(2.63-4.67)**
BMI (Kg/m^2)				
20.0-24.99 (normal weight)	2,676(38.5)	852(38.5)	362(41.0)	1.00
<20.00 (underweight)	425(6.1)	110(4.95)	61 (6.9)	1.12(0.80-1.57)
25.0-29.9 (overweight)	2,110(30.3)	732(33.1)	310 (35.1)	1.06(0.89-1.27)
> 30.0 (obese)	1,749(25.1)	521(23.5)	151(17.1)	**0.65(0.52-0.80)**
Osteoporosis				
No	6,245(89.2)	1,718(77.4)	766(86.6)	1.00
Yes	753(10.8)	503(22.6)	118(13.4)	0.98(0.79 -1.22)
Hypertension				
No	4,976(71.0)	1,260(56.6)	614(69.3)	1.00
Yes	2,045(29.1)	965(43.4)	272(30.7)	0.92(0.78-1.08)
Diabetes				
No	6,105(87.0)	1,888(84.7)	796(90.0)	1.00
Yes	915(13.0)	341(15.3)	89(10.1)	**0.71(0.55-0.91)**
Cardiovascular disease				
No	6,434(91.8)	1,922(86.5)	819(92.8)	1.00
Yes	577(8.2)	301(13.6)	63(7.2)	**0.74(0.56-0.97)**
Breast cancer				
No	6,678(95.1)	2,082(93.4)	864(97.5)	1.00
Yes	348 (4.9)	146(6.6)	22(2.5)	**0.46(0.28-0.76)**
Ovarian cancer				
No	6,989(99.5)	2,207(99.0)	880(99.3)	1.00
Yes	37(0.5)	22(0.9)	6 (0.7)	1.20(0.48-2.60)
Other female cancers				
No	6,887(98.0)	2,165(97.1)	863(97.4)	1.00
Yes	139 (2.0)	63(2.9)	23(2.6)	1.19(0.68 -2.07)
Allergies				
No	4,009(57.2)	1,195(53.7)	436(49.3)	1.00
Yes	2,999(42.8)	1,030(46.3)	449(51.0)	**1.33(1.14-1.55)**
Mood Disorders				
No	5,906(84.1)	1,794(80.5)	656(74.0)	1.00
Yes	1,115(15.9)	434(19.5)	230(26.0)	**1.75(1.46-2.09)**
Anxiety Disorders				
No	6,417(91.4)	2,017(90.6)	790(89.2)	1.00
Yes	601(8.6)	209(9.4)	95(10.8)	1.25 (0.98-1.61)

[a] Frequencies are column percentages estimated using analytic weights.

[b] Multinomial logistic regression model predicting HT current vs. never use: odds ratio >1 indicates a higher likelihood of being a current HT user, results of past vs. never use are not shown.

Table 4.3: Results of the adjusted multinomial logistic analysis for current HT use.

	Current [a]
	Adjusted OR (95%CI)
Variables	
Sociodemographic	
Age (years)	
<50	1.00
50-59	1.28(0.86-1.90)
60-69	1.32(0.83-2.10)
70-79	0.87(0.52-1.45)
≥80	**0.31(0.15-0.63)**
Ethnicity	
White (non-aboriginal)	1.00
Aboriginal	0.96(0.60-1.52)
Mixed/other	**0.32(0.14-0.73)**
Marital Status	
With partner	1.00
No partner	0.81(0.65-1.01)
Education Level	
Less than high school	1.00
High school to some college	1.08(0.79-1.47)
Bachelor's degree or higher	1.02(0.73-1.42)
Employment Status	
Not currently working	1.00
Currently working	**0.76(0.61-0.94)**
Household Income (CAD)	
<20,000	1.00
20,000-50,000	0.82(0.57-1.18)
50,000-100,000	1.15(0.78 -1.69)
100,000-150,000	1.15(0.74-1.80)
>150,000	1.41(0.87-2.29)
Lifestyle	
Smoking Status	
Never	1.00
Former	1.11(0.92-1.34)
Current	**0.69(0.49-0.97)**
Alcohol consumption	
Never	1.00
Drinks less than weekly	**1.51(1.13-2.02)**
Drinks at least weekly	**1.94(1.44-2.61)**
Leisure time physical activity	
Non-regular	1.00
Regular	**1.23(1.03-1.46)**

Table 4.3 (Cont'd): Results of the adjusted multinomial logistic analysis for current HT use.

Health related	
Menopause Status	
No	1.00
Natural	**3.01(2.11-4.30)**
Surgical (Hysterectomy)	**7.33(5.05-10.6)**
BMI (Kg/m^2)	
20.0-24.99 (normal weight)	1.00
<20.00 (underweight)	1.09(0.74-1.60)
25.0-29.99 (overweight)	1.05(0.86-1.27)
> 30.0 (obese)	**0.57(0.44-0.75)**
Osteoporosis	
No	1.00
Yes	1.08(0.83-1.39)
Hypertension	
No	1.00
Yes	1.10(0.90-1.35)
Diabetes	
No	1.00
Yes	0.84(0.63-1.12)
Cardiovascular disease	
No	1.00
Yes	0.84(0.61-1.17)
Breast cancer	
No	1.00
Yes	**0.43(0.26-0.71)**
Ovarian cancer	
No	1.00
Yes	1.10(0.40-3.01)
Other female cancers	
No	1.00
Yes	1.13(0.59-2.14)
Allergies	
No	1.00
Yes	**1.29(1.08-1.54)**
Mood Disorders	
No	1.00
Yes	**1.86(1.50-2.32)**
Anxiety Disorders	
No	1.00
Yes	1.21(0.89-1.64)

[a] Multinomial logistic regression model predicting HT current vs. never use: odds ratio >1 indicates a higher likelihood of being a current HT user, results of past vs. never use are not shown.

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Chapter 5
General Discussion

This dissertation examined the following objectives using data from the Canadian Longitudinal Study on Aging (CLSA) and the Coronary Artery Risk Development in Young Adults (CARDIA) study: Objective (1): To assess the impact of changes in weight, BMI, and waist circumference across time on ANM in a sample of US women. Objective (2): To elucidate median ANM and investigate associations between various factors, and ANM among Canadian women. Objective (3): To determine the prevalence of HT use and evaluate the associations between various factors, and HT use among Canadian women. Below is a description of the major findings by chapter.

5.1 Major Findings

In chapter 2, baseline WC was associated with a later ANM. Neither absolute nor relative changes in anthropometric measures were associated with ANM with one exception. If there was more than 5% weight loss since baseline, the hazard for an earlier ANM decreased by 45%. Premenopausal hypertension was strongly associated with an earlier ANM, highlighting the importance of cardiovascular risk factors on ANM. In chapter 3, median ANM was 51 years. Having no partner, low household income and education levels, current and former smoking, and cardiovascular disease (CVD) were associated with an earlier ANM, while current employment, any alcohol consumption, and obesity were associated with later ANM. Finally, in chapter 4, using data from the CLSA, we found that 9.5% of women had reported current HT use. The factors negatively associated with current HT use were: older age, non-white ethnic background, current employment, regular smoking, obesity, and breast cancer. By contrast, alcohol consumption, and the presence of allergies or mood disorders were positively associated with current HT use.

5.2 Public Health Implications

Natural menopause is a complex bio cultural phenomenon with geographic and ethnic variations. ANM is a risk factor for a number of chronic conditions. Therefore, understanding its

factors help can identify menopausal women who are at high risk for future morbidity. Despite increasing evidence of the importance of natural menopause as a sentinel for future health outcomes, no research has examined its associations with various socioeconomic, lifestyle and clinical factors in the Canadian population. In addition, limited research has accounted for the life course aspect of body size in relation to age at menopause. Using a life course framework, Chapter 2 provided insight into potential points of intervention during early adulthood which could possibly break the series of cardiovascular risks accumulating over time and thus help reduce the associated later health outcomes of ANM. This dissertation used novel techniques by examining the influence of various factors on HT use and ANM, and has resulted in findings that would benefit the fast-growing menopausal population. HT is currently the most effective therapy in managing the symptoms that occur during the menopause transition[39-41]. HT use rates have declined in the years after the publication of several randomized trials in older women that suggested coronary harm and that the risks involved, such as breast cancer, outweighed any benefit. However, important recent statements from the authors of the WHI and the International Menopause Society have emerged stating that, after a personalized risk assessment, an appropriate HT formulation is safe, and with a more favorable benefit–risk ratio among young postmenopausal women[44]. The landscape of menopause management has changed in the past 20 years. Therefore, elucidating the prevalence of HT use and the extent to which individual sociodemographic, lifestyle, and clinical characteristics are associated with HT use, may inform clinicians about women who are not using HT but perhaps should be using it. Indeed, Chapter 4 showed that women with obesity are less likely to be current HT users.

Estrogen deficiency promotes metabolic dysfunction predisposing women to MetS and type 2 diabetes. Given an established relationship between obesity and CVD risk factors, estrogen's effect on both of these processes is of importance in menopause treatment. In fact, large randomized controlled trials have suggested that HT reduces incidence of type 2 diabetes in women[63, 64], and as a consequence, might reduce CVD risk. Promoting early preventive strategies for weight management and clinical monitoring of cardiovascular risk factors for women in the menopause transition constitute an important point of intervention for reducing future CVD burden[65]. Smoking is also a strong predictor of early natural menopause, as shown in chapter 2. Reducing early menopause by eliminating smoking could provide an additional

benefit from smoking cessation, although there is no available evidence that smoking cessation extends menopausal age. Our findings further provided evidence that CVD and hypertension account for an earlier ANM, as seen in chapters 2 and 3. It is possible that CVD risk early in life contributes to both early menopause and CVD via similar mechanisms. Trajectories of cardiovascular risk factors (such as cholesterol, weight, and blood pressure) measured during the pre-menopause have been associated with the timing of subsequent natural menopause in one study[66]. Using a life course framework, the effect of body size at early adulthood affected ANM. As a result, we also provided insight into potential points of intervention specifically during early adulthood and which could possibly break the series of risks accumulating over time and thus reduce the associated health effects of ANM. Accordingly, the findings of the current dissertation fill an important gap in the literature.

5.3 Limitations

There are a few limitations in the three analyses that are worth noting. The cross-sectional nature of the CLSA study precludes inference of causality. Second, the potential for reverse causality in some variables is probable; for example, some women might choose to start exercising after initiating HT. Recall bias due to self-report of HT use as well as ANM might also be another limitation. Also, the exclusion of women with surgical menopause might affect the generalizability of findings. Second, the self-selected nature of participants in the CARDIA, as well as the exclusions made for the present analysis might limit generalizability of findings. The data were also subject to right censoring because participants had "survived" (not yet postmenopausal). However, our analyses accounted for entry at ages 40 years and higher.

5.4 Strengths

This dissertation leveraged two existing national, large datasets with a wealth of information on important risk factors for ANM and HT use. Strengths include that the second and last analyses were the first to present a detailed description of HT use and ANM in Canada. In addition, use of a large and nationally representative sample from the CLSA Tracking Cohort allowed generalizability of our findings to the majority of the Canadian population. Furthermore, the simultaneous adjustment of multiple factors in the Cox model, while censoring at age of HT initiation, limited the potential for confounding bias. There were repeated measures on health-

related risk factors in the CARDIA which were accordingly modeled as time-varying covariates. Moreover, availability of data (CARDIA) with over a decade of follow-up provided a unique opportunity to understand the associations between earlier life course body size and age at menopause in mid-life, since its design helps establish temporality between exposure and outcome.

5.5 Future Research Directions

First, longitudinal analysis of the CLSA tackling the same objective in Chapter 3 is needed to confirm the findings obtained. Stratification of BMI by obesity classes may show different associations with ANM and so warrant further investigation. In light of the importance of cardiovascular risk factors in the pathway to ANM, as shown in this research, future studies should plan to examine inflammatory markers and subclinical measures of CVD, such as carotid intima media thickness (ideally with repeated measurements to assess change across time) and changes in sex hormone levels as pathways between menopause onset and cardiovascular health and mortality later in life. This research would provide further insight into the timing of interventions needed during this period and help translate epidemiologic data into effective policies and solutions. Additional prospective studies on dietary factors such as fat and protein intake and ANM are necessary to provide a conclusive answer on their relationship. Studies are required to determine the causal association between CVD, as well as the mediating role of lifestyle factors, such as physical activity and alcohol use, and type of menopause and ANM by using longitudinal techniques. Future research should aim to prospectively examine the prevalence of HT use according to type of HT and menopause type, since our research used cross sectional data. The relationships between current employment, alcohol use, and HT use remain unclear and warrant further investigation, as Chapter 4 revealed. The role of HT use among women with atopy and asthma, as well as the intersection of obesity in this relationship is unclear, since this gap has emerged in our results. Finally, more work addressing similar research questions as this dissertation among minority or socially disadvantaged groups, such as Indigenous peoples, while also accounting for the role of sex hormones, is needed to replicate our findings.

5.6 Conclusion

With the increase in life expectancy, the proportion of postmenopausal life also increases. ANM is critical for a woman's health trajectory, as it is an indicator of ovarian function and aging. Our results highlighted that menopausal age is associated with modifiable factors. Findings might inform prevention strategies that benefit many women going through the menopausal transition, highlighting the importance of both control and prevention of cardiovascular risk factors, especially blood pressure, in adulthood prior to menopause when cardiovascular risk factors are prone to change. Its findings may also be used to inform opportunities for improved physician–patient communication regarding menopause management, and help in risk assessment, prevention and early management of chronic disease risk during the menopausal transition. Therefore, the need for primary and secondary prevention of cardiovascular risk factors among women at midlife is a pressing one. Menopause may represent a state that requires clinical recognition and specific appropriate treatment. The increased risks of HT demonstrated in the WHI had profound effects on clinical practice. Therefore, ensuring that practicing clinicians and trainees are equipped with current evidence on menopause management and available treatment options is integral to individualizing treatment. Policies stating that physicians completing residencies in areas related to menopause receive core competencies and updates on treatment modes need to be developed and implemented. Moreover, it is important for women to share in decision-making about their symptom management while having the support of a healthcare system that is also well-informed on all aspects of this stage in their lives.

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Appendix A:

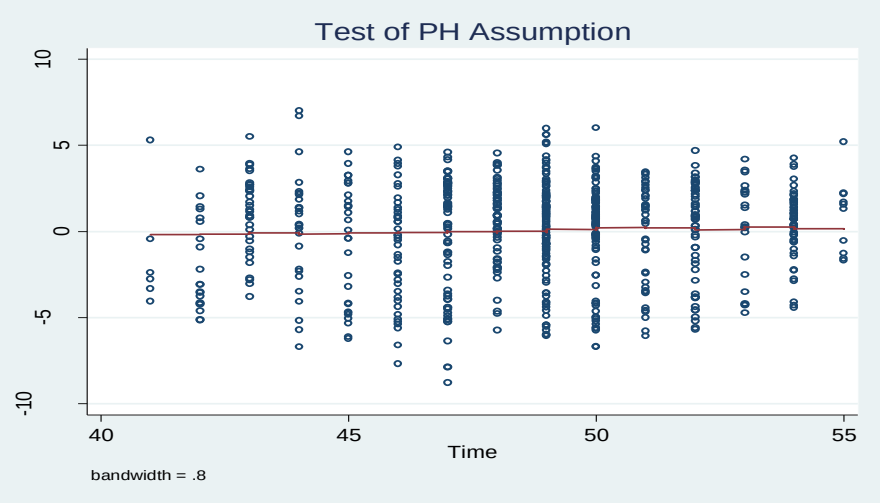
In the **stphtest** command, the proportionality of the model as a whole is tested, and by using the **detail** option, a test of proportionality for each predictor is obtained. If the tests in the table are not significant, then proportionality cannot be rejected and there is no violation of the proportional assumption. By using the plot option a graph of the scaled Schoenfeld assumption can be obtained. A horizontal line in the graphs is further indication that there is no violation of the proportionality assumption. The assumption of proportional hazards for each analysis was checked both by inspection plots and Schoenfeld residuals, which did not indicate violation of the assumption.

PH assumption tests examples, CARDIA:
quietly stcox race educ inc smk wrk pa bmi bmibaseline glu logins diabet htn sbp dbp trg ldl hdl chol hchol logbn_tfat logbn_carbo logbn_prot , schoenfeld(sch*) scaledsch(sca*)
stphtest, detail

```
Test of proportional-hazards assumption

Time:  Time
```

	rho	chi2	df	Prob>chi2
race	0.07937	4.86	1	0.0276
educ	0.01162	0.09	1	0.7633
inc	0.00083	0.00	1	0.9828
smk	-0.07280	3.80	1	0.0513
wrk	0.01036	0.07	1	0.7902
pa	0.04915	1.75	1	0.1862
bmi	-0.06667	3.20	1	0.0735
bmibaseline	0.05564	2.35	1	0.1251
glu	-0.02936	0.48	1	0.4904
logins	0.06884	3.35	1	0.0674
diabet	-0.01191	0.10	1	0.7525
htn	0.02638	0.47	1	0.4943
sbp	-0.07017	3.55	1	0.0596
dbp	0.07755	4.78	1	0.0289
trg	-0.01323	0.12	1	0.7245
ldl	-0.01704	0.21	1	0.6491
hdl	-0.01530	0.17	1	0.6829
chol	0.01679	0.20	1	0.6541
hchol	-0.01541	0.16	1	0.6920
logbn_tfat	0.06177	2.57	1	0.1087
logbn_carbo	-0.00171	0.00	1	0.9652
logbn_prot	-0.03177	0.81	1	0.3672
global test		30.79	22	0.1005

stphtest, plot(race) msym(oh)

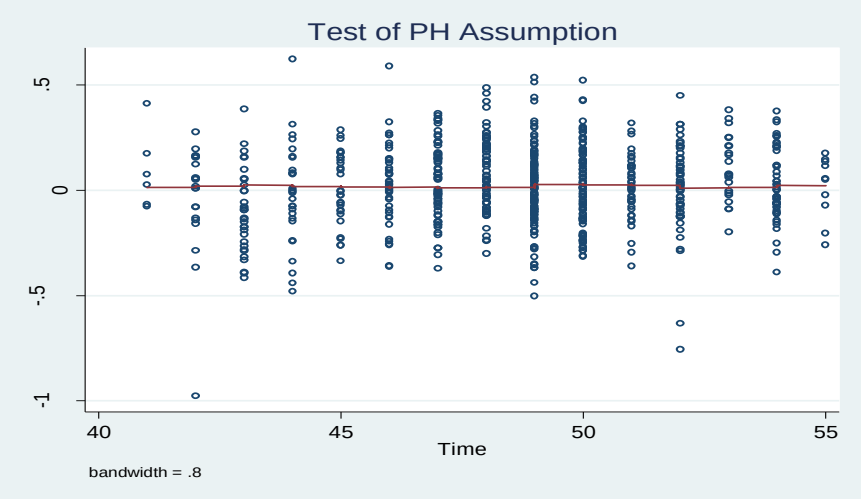


quietly stcox race educ inc smk wrk pa wgt wgtbaseline glu logins diabet htn sbp dbp trg ldl hdl chol hchol logbn_tfat logbn_carbo logbn_prot , schoenfeld(sch*) scaledsch(sca*)
stphtest, detail

```
Test of proportional-hazards assumption

Time:  Time
```

	rho	chi2	df	Prob>chi2
race	0.07937	4.86	1	0.0276
educ	0.01162	0.09	1	0.7633
inc	0.00083	0.00	1	0.9828
smk	-0.07280	3.80	1	0.0513
wrk	0.01036	0.07	1	0.7902
pa	0.04915	1.75	1	0.1862
bmi	-0.06667	3.20	1	0.0735
bmibaseline	0.05564	2.35	1	0.1251
glu	-0.02936	0.48	1	0.4904
logins	0.06884	3.35	1	0.0674
diabet	-0.01191	0.10	1	0.7525
htn	0.02638	0.47	1	0.4943
sbp	-0.07017	3.55	1	0.0596
dbp	0.07755	4.78	1	0.0289
trg	-0.01323	0.12	1	0.7245
ldl	-0.01704	0.21	1	0.6491
hdl	-0.01530	0.17	1	0.6829
chol	0.01679	0.20	1	0.6541
hchol	-0.01541	0.16	1	0.6920
logbn_tfat	0.06177	2.57	1	0.1087
logbn_carbo	-0.00171	0.00	1	0.9652
logbn_prot	-0.03177	0.81	1	0.3672
global test		30.79	22	0.1005

stphtest, plot(dbp) msym(oh)



quietly stcox race educ inc smk wrk pa wst wstbaseline glu ins diabet htn sbp dbp trg ldl hdl chol hchol logbn_tfat logbn_carbo logbn_prot , schoenfeld(sch*) scaledsch(sca*)
stphtest, detail

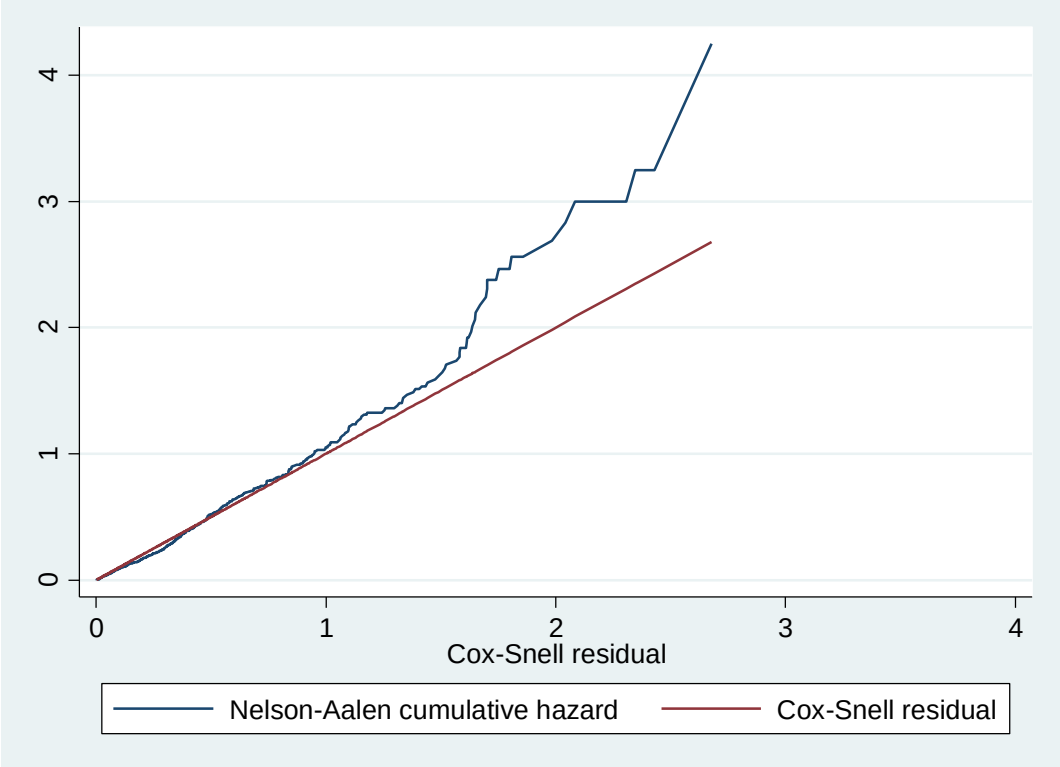
```
Test of proportional-hazards assumption

Time:  Time
```

	rho	chi2	df	Prob>chi2
race	0.07937	4.86	1	0.0276
educ	0.01162	0.09	1	0.7633
inc	0.00083	0.00	1	0.9828
smk	-0.07280	3.80	1	0.0513
wrk	0.01036	0.07	1	0.7902
pa	0.04915	1.75	1	0.1862
bmi	-0.06667	3.20	1	0.0735
bmibaseline	0.05564	2.35	1	0.1251
glu	-0.02936	0.48	1	0.4904
logins	0.06884	3.35	1	0.0674
diabet	-0.01191	0.10	1	0.7525
htn	0.02638	0.47	1	0.4943
sbp	-0.07017	3.55	1	0.0596
dbp	0.07755	4.78	1	0.0289
trg	-0.01323	0.12	1	0.7245
ldl	-0.01704	0.21	1	0.6491
hdl	-0.01530	0.17	1	0.6829
chol	0.01679	0.20	1	0.6541
hchol	-0.01541	0.16	1	0.6920
logbn_tfat	0.06177	2.57	1	0.1087
logbn_carbo	-0.00171	0.00	1	0.9652
logbn_prot	-0.03177	0.81	1	0.3672
global test		30.79	22	0.1005

Goodness of fit of the model can be evaluated by using the Cox-Snell residuals. If the model fits the data well then the true cumulative hazard function conditional on the covariate vector has an exponential distribution with a hazard rate of one. This translates into fitting the model using the **stcox** command and specifying the **mgale** option which will generate the martingale residuals. Then the **predict** command with the **csnell** option is employed to generate the Cox-Snell residuals for the model. The data is reset using the **stset** command specifying the variable **cs**, the variable containing the Cox-Snell residuals, as the time variable. The **sts generate** command is used to create the Nelson-Aalen cumulative hazard function. Finally, the Nelson-Aalen cumulative hazard function and the cs variable are graphed so that the hazard function can be compared to the diagonal line. If the hazard function follows the 45 degree line then it approximately has an exponential distribution with a hazard rate of one and that the model fits the data well. Goodness of fit of the multivariate Cox models was assessed using the likelihood ratio test and Cox-Snell residuals, which indicated a good overall fit.

Goodness of fit example, CARDIA:

```
quietly stcox race educ wrk inc smk drnk bc parity pac wst wstbaseline glu logins diabet htn sbp
dbp trg ldl hdl chol hchol logbn_tfat logbn_carbo  logbn_prot , nohr mgale(mg)
predict cs, csnell
stset cs, failure(failure)
sts generate H = na
line H cs cs, sort xlab(0 1 to 4) ylab(0 1 to 4)
```



Appendix B:

In the **stphtest** command, the proportionality of the model as a whole is tested, and by using the **detail** option, a test of proportionality for each predictor is obtained. If the tests in the table are not significant, then a violation of the proportional assumption is not present. The **stphplot** command uses log-log plots to test proportionality and if the lines in these plots are parallel then we have further indication that the predictors do not violate the proportionality assumption. Validity of the proportional hazards assumption was checked by log-log plots with most variables not violating the assumption with the exception of: household income, smoking status, and CVD status. However, upon close inspection of the plots, the curves were close to parallel, with a minimal effect on the interpretation of the results. Moreover, based on prior research those variables were important factors, therefore the model was left unaltered.

PH assumption test, CLSA:

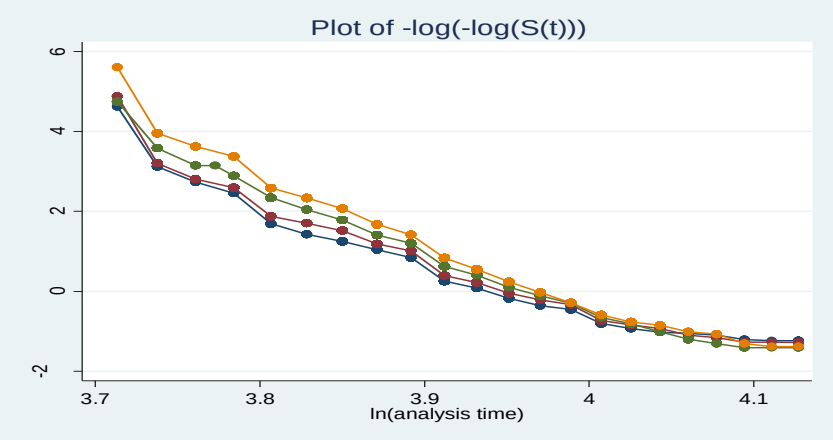
quietly stcox eth_fin_white income_rec marital_stat_recoded educ_rec current_wrk smk_status alc_freq_recoded sport_part bmi_recoded allergies_rec htn_rec diab_rec CVD_no anx_rec mooddis_rec , schoenfeld(sch*) scaledsch(sca*)

stphtest, detail

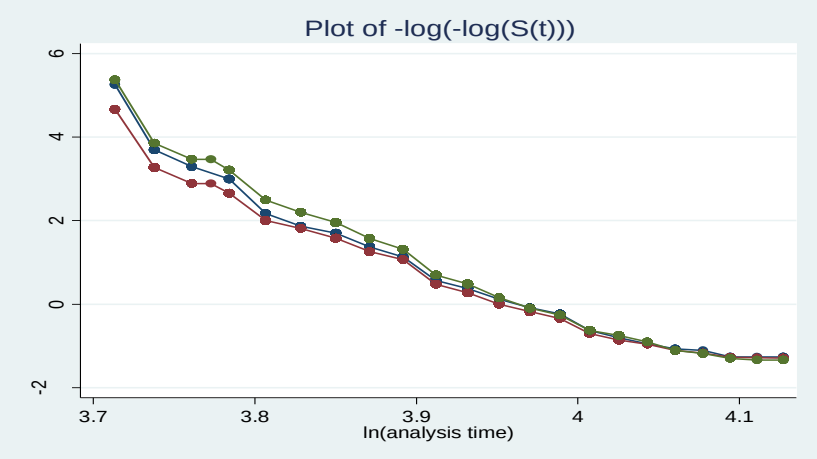
```
Test of proportional-hazards assumption

Time:  Time
```

	rho	chi2	df	Prob>chi2
eth_fin_wh~e	0.00651	0.20	1	0.6547
income_rec	0.04037	7.71	1	0.0055
marital_st~d	-0.02261	2.41	1	0.1202
educ_rec	0.03100	4.34	1	0.0373
current_wrk	0.05275	13.35	1	0.0003
smk_status	-0.06034	17.76	1	0.0000
alc_freq_r~d	0.01712	1.39	1	0.2378
sport_part	0.01268	0.76	1	0.3835
bmi_recoded	0.00418	0.08	1	0.7728
allergies_~c	0.01063	0.53	1	0.4663
htn_rec	-0.00993	0.46	1	0.4972
diab_rec	-0.01034	0.50	1	0.4789
CVD_no	0.03800	6.74	1	0.0094
anx_rec	0.00153	0.01	1	0.9161
mooddis_rec	0.01050	0.53	1	0.4684
global test		108.93	15	0.0000

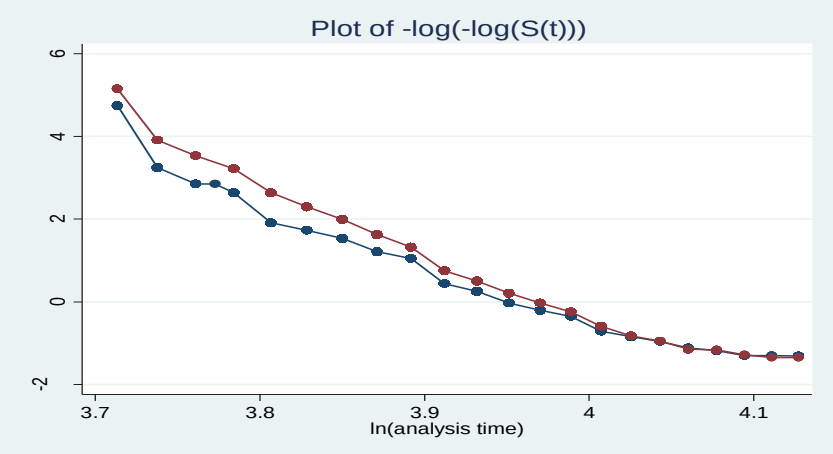
stphplot, by(income_rec) legend(off) title(Plot of -log(-log(S(t))))
graph export coxphplot.png, width(500) replace



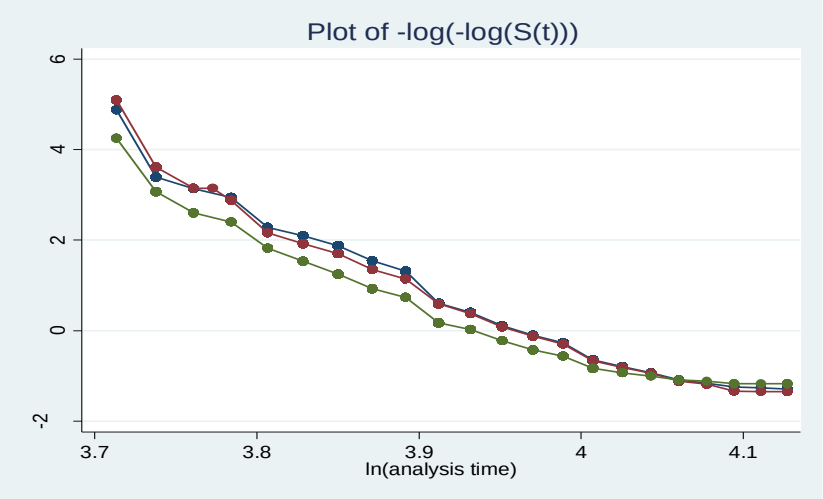
stphplot, by(educ_rec) legend(off) title(Plot of -log(-log(S(t))))
graph export coxphplot.png, width(500) replace



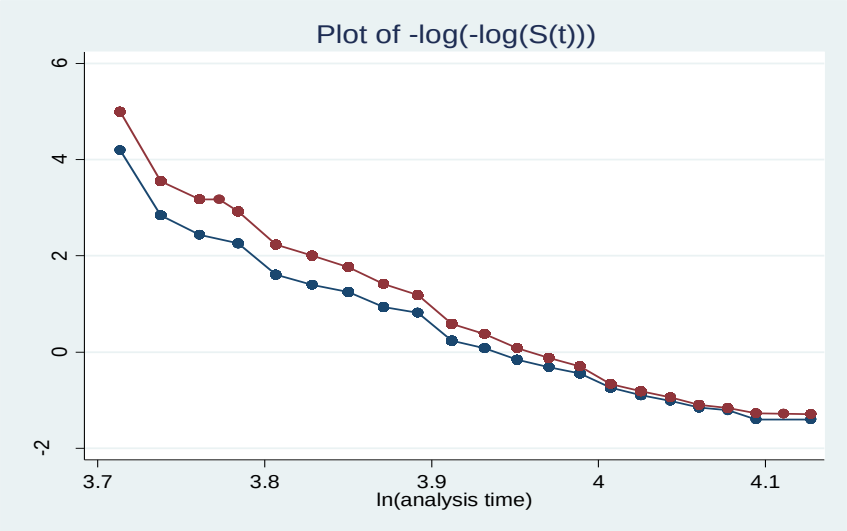
stphplot, by(current_wrk) legend(off) title(Plot of -log(-log(S(t))))
graph export coxphplot.png, width(500) replace



stphplot, by(smk_status) legend(off) title(Plot of -log(-log(S(t))))
graph export coxphplot.png, width(500) replace



stphplot, by(CVD_no) legend(off) title(Plot of -log(-log(S(t))))
graph export coxphplot.png, width(500) replace



Goodness of fit of the model can be evaluated by using the Cox-Snell residuals. If the model fits the data well then the true cumulative hazard function conditional on the covariate vector has an exponential distribution with a hazard rate of one. This translates into fitting the model using the **stcox** command and specifying the **mgale** option which will generate the martingale residuals. Then the **predict** command with the **csnell** option is employed to generate the Cox-Snell residuals for the model. The data is reset using the **stset** command specifying the variable **cs**, the variable containing the Cox-Snell residuals, as the time variable. The **sts generate** command is used to create the Nelson-Aalen cumulative hazard function. Finally, the Nelson-Aalen cumulative hazard function and the cs variable are graphed so that the hazard function can be compared to the diagonal line. If the hazard function follows the 45 degree line then it approximately has an exponential distribution with a hazard rate of one and that the model fits the data well. Goodness of fit of the multivariate Cox model was also assessed using the likelihood ratio test and Cox-Snell residuals to create the Nelson-Aalen cumulative hazard function. Our data overall followed an exponential distribution indicating that our model fits the data well.

Goodness of fit test, CLSA:

```
quietly stcox eth_fin_white marital_stat_recoded educ_rec current_wrk income_rec smk_status alc_freq_recoded
sport_part bmi_recoded htn_rec allergies_rec diab_rec CVD_no mooddis_rec anx_rec, nohr mgale(mg)
. predict cs, csnell
. stset cs, failure(failure)
sts generate H = na
line H cs cs, sort xlab(0 1 to 4) ylab(0 1 to 4)
```